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The Synthetic Organic Hypnotics*,†

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HISTORICAL

The principle of the production of sleep through administration of drugs was discovered many centuries ago when the physiological actions of ethyl alcohol and opium became known. Progressive developments in the field of hypnotics, however, might be considered as beginning with the discovery of the action of chloral by Liebreich in 1869. Although the hypnotic properties of the inorganic bromides were demonstrated some five years before this date, the bromides are more correctly classified as sedatives since doses sufficient to cause hypnosis too often will produce marked toxic reactions.

From the time of the discovery of the action of chloral until 1903 when Fischer and von Mering introduced barbital, three other useful hypnotics, namely, paraldehyde, urethane and sulfonal, were added to the list.

It is interesting to note that these compounds have little in common so far as their chemical structures are concerned. Furthermore, they serve as examples of the chemical classes into which fall nearly all of the therapeutically important hypnotics in current use. Intensive studies, carried out for the most part during the past twenty-five years, have greatly increased our knowledge concerning the changes in therapeutic action brought about by alteration in chemical structure of the parent compounds discovered between 1869 and 1903. Little or no improvement in the physiological properties of chloral, paraldehyde, urethane or sulfonal has resulted from this work.

Investigations of a similar nature in the case of barbital, however, have led to the development of a whole series of new hypnotics. The members of this group, most of which are unsymmetrical 5,5′-dialkylbarbituric acids, have proved to be sufficiently satisfactory in clinical use so that other types of hypnotics have assumed a position of secondary importance.

Many organic compounds which belong to classes other than those already described have been shown to possess hypnotic properties but with few exceptions they have not found any extensive use in medicine. They are important mainly because a knowledge of their properties has served to extend the scope of the entire field of hypnotics,

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especially in relating chemical structure to physiological activity.

GENERAL CONSIDERATIONS

The ideal hypnotic should have properties such that, when administered in proper dose, it will cause a rather prompt but mild depression of the central nervous system sufficient to reduce perception of external stimuli and thus allow a condition of natural sleep to follow. The action should persist for several hours and there should be no undesirable secondary effects, for example, preexcitement, after-depression, cumulative toxicity, and depression of such functions as respiration and blood pressure. A drug with all these characteristics has not been developed but a number of hypnotics are available which have a minimum of undesirable properties.

A hypnotic given in small doses serves as a suitable sedative where a calming action without sleep is required. In other instances hypnotics may be used to produce general anesthesia although their value for such a purpose is limited, mainly because they are compounds of relatively low volatility and, hence, must be given by some means other than inhalation. This makes it impracticable to administer them in the small graduated doses possible with a volatile anesthetic such as ether. The use of hypnotics is also limited to the treatment of conditions in which pain is absent, because a hypnotic lacks analgesic properties and its administration in the presence of pain may produce delirium. Hypnotics are employed mainly as symptomatic agents in the relief of insomnia and for the prevention or relief of preoperative apprehension.

The mechanism by which the hypnotics bring about the desired type of depression of the central nervous system is not well understood although it has been the subject of much study. It is known, however, that certain physical properties of compounds causing hypnosis, such as their lipoid solubility and surface affinity, are more directly involved than are the chemical properties. Since compounds widely diversified in structure, for example, alcohols, sulfones, chlorinated aldehydes and barbituric acids, all produce similar physiological responses, hypnosis probably arises from the action of the molecule as a whole and not by virtue of the presence of any particular chemical grouping it may contain. In general, the introduction of certain radicals, for example, alkyl and chlorine, will enhance but not initiate the hypnotic action of the parent substance.

Many theories have been proposed to explain the mechanism of hypnotic action but most of these have been discarded as untenable in the light of closer investigation. The so-called "Meyer-Overton Law," which states that there is a direct relationship between the depressant action and lipoid solubility of a substance, seems to coincide with many of the observed phenomena of narcosis. According to this concept, the greater the ratio of the solubility of a compound in oil to its solubility in water, the more potent is the narcotic. Exceptions to this theory are sufficiently numerous, however, to make it apparent that other factors are involved. It is probable that hypnotics may act through more than one mechanism. The theories of the mechanism of narcotic action have been ably discussed by Sollmann (1*a*).

CHEMICAL CLASSIFICATION OF HYPNOTICS

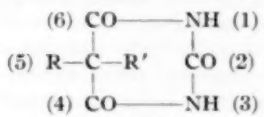
Classification of hypnotics into groups according to their chemical structure for the purpose of correlating hypnotic potency with the atomic arrangement of the molecules in question is of limited value as a guide for the investigator seeking to develop better hypnotics. The conclusions that can be reached from studying such an arrangement are relatively few and those generalizations which can be made are too indefinite in nature to serve as a satisfactory index for further investigation. Shonle (2) has pointed out that, in general, a hypnotic consists of a fat-soluble alkyl group in combination with a water-soluble radical capable of forming associated molecules in aqueous solvents. Although it is usually necessary for a compound to contain this combination in order that it may possess hypnotic activity, it does not follow that all chemicals so constituted are hypnotics. Many hypnotics contain one or more chlorine or bromine atoms but the mere presence of one of the halogens is not indicative of an ability to produce hypnosis. It may, therefore, be said that, for a substance to be a hypnotic, certain arrangements of the atoms within a molecule are essential but the presence of such groupings is no guarantee of a positive hypnotic action. Consequently, these postulates find their greatest use in enabling one to segregate those compounds which are most likely to possess hypnotic properties.

Although a system of classification based upon chemical structure has been used below for purposes of discussion, and frequent mention is made of the relations existing between structure and hypnotic activity, it should be understood that an arrangement based upon relative physical properties

would present a picture more closely associated with the actual factors which influence potency. Changes in chemical composition probably affect the degree of activity mainly because of the concomitant alteration in physical properties. Until we have a fuller knowledge of the mechanism of hypnotic action, and until a systematic measurement of the properties influencing this mechanism has been made, we are obliged to use the only available substitute—chemical classification.

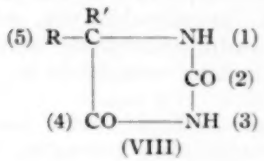
The types of organic compounds enumerated below include nearly all the useful hypnotics. Each class will be discussed briefly and a short description of the properties of some of the more important members is included.

Alcohols — ROH (I)
Aldehydes — RCHO (II)
Acid amides — $RCONH_2$ (III)
Acyl ureas — $RCONHCONH_2$ (IV)
Alkyl ureas — $RNHCONH_2$ (V)
Alkyl carbamates — H_2NCOOR (VI)
Barbituric acids —



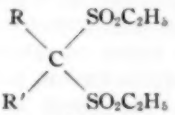
(VII)

Hydantoins—



(VIII)

Sulfones—



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DISCUSSION

Alcohols.—All the aliphatic alcohols so far examined possess hypnotic properties and it was recognized as early as 1869 (3) that this action becomes more pronounced with increasing molecular weight. Both the activity and toxicity of the normal primary alcohols increase until the molecule contains six to eight carbon atoms; beyond this point the potency falls off, until alcohols containing about sixteen carbon atoms are inert (4). Hypnotic potency becomes greater as the alcohol chain becomes more branched; thus, in a series of three alcohols containing the same number of carbon atoms, the activity increases in the order: primary, secondary and tertiary.

Ethyl alcohol and tertiary amyl alcohol are the only unsubstituted alcohols that are used to any extent for their somnifacient properties. The systemic action of ethyl alcohol is primarily depression of the nervous system. It is useful as a sedative and hypnotic when taken in the absence of excitement. However, its continued use for this purpose is dangerous because of the possibility of inducing chronic alcoholism (1*b*). Tertiary amyl alcohol was formerly employed to some extent as a sedative and hypnotic, its activity ranking between chloral and paraldehyde. The development of other more satisfactory hypnotics has practically eliminated its use except as a solvent and synergist as described below.

Introducing three chlorine or bromine atoms onto the same carbon is a highly efficient means of increasing the hypnotic activity of an alcohol and the effects seem to be about equally pronounced whether the alcohol be a straight or a branched chain compound. Tribromoethanol is many times more potent than ethyl alcohol and it is used rather extensively as a basal anesthetic, administered rectally. Its main value lies in the fact that it rapidly produces sleep with no pre-excitement and, when used in combination with ether, excellent muscular relaxation is obtained. Tribromoethanol is a solid which is unstable in aqueous solution. It is marketed in liquid form as a solution with tertiary amyl alcohol under the name of "Avertin with Amylene Hydrate." This preparation is dissolved in about forty volumes of water and administered as the freshly prepared solution. Tertiary amyl alcohol modifies the action of tribromoethanol to some extent because of its own hypnotic action. It also increases the depth and duration of hypnosis as well as adding to the respiratory depression (1*c*). When used with proper care, tribromoethanol is a very satisfactory agent for inducing basal anesthesia.

Trichloroethanol is quite similar in action to tribromoethanol although it seems to cause less respiratory depression (5, 6, 7). It has been used in a limited clinical trial (8) where frequent occurrence of excitement and tachycardia were observed. Results obtained so far in comparing the two halogenated ethanols indicate that the trichloro derivative is not sufficiently different in action to warrant its use in the clinic.

Trichloroisopropyl alcohol or "Isopral" is an example of a secondary alcohol whose hypnotic properties have been greatly enhanced by chlorination. This chlorinated compound is said to be about twice as active as chloral but clinical results have been un-

certain and its use was found to be dangerous.

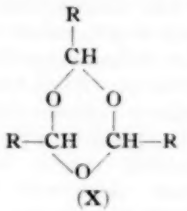
Trichlorotertiary butyl alcohol, better known as Chlorobutanol or Chloretone, is another halogenated alcohol possessing powerful hypnotic properties and behaving similarly to chloral in its physiological action. Chlorobutanol is sometimes used as a preoperative hypnotic and it is frequently the anesthetic of choice for laboratory animals because it allows long operations without requiring further attention to maintain anesthesia. Chlorobutanol also possesses useful local anesthetic and bacteriostatic properties which make it valuable for preparing various pharmaceuticals such as nasal sprays and parenteral solutions.

Aldehydes.—The simple aliphatic aldehydes produce hypnotic effects upon experimental animals although, in general, the doses required are sufficient to cause simultaneous irritation and other serious toxic reactions. Chlorination of aliphatic aldehydes increases their hypnotic activity and alters their irritating properties to such an extent that certain members of the group become therapeutically useful.

Chloral or trichloroacetaldehyde, usually dispensed as the hydrate, is an example. Chloral was first used by Liebreich with the idea that it would decompose into chloroform in the body and induce anesthesia. Although the expected type of decomposition did not occur, a valuable hypnotic was discovered which continued to be the drug of choice for many years. Since the introduction of more satisfactory hypnotics, chloral has become less important, principally because the newer compounds induce fewer simultaneous secondary reactions (for example, gastric irritation, depression of respiration, vasomotor paralysis, and depression of the cardiac muscle) which are fairly common after chloral administration. Chloral is particularly useful in cases of simple insomnia where a small dose of the drug is sufficient to produce a prompt, natural sleep which lasts for several hours. It has been used to combat conditions of excessive nerve stimulation such as the convulsions from strychnine and tetanus.

In view of the fact that substitution with three atoms of chlorine in the acetaldehyde or the ethyl alcohol molecule produces derivatives with pronounced hypnotic action and since the activities of trichloro- and tribromoethanol are so similar, tribromoacetaldehyde would be expected to behave in an analogous manner. This is not the case, however; Lehmann and Knoefel (7) have found that tribromoacetaldehyde in one-tenth the hypnotic dose of chloral hydrate produces excitement followed by apathy, muscular weakness and cyanosis. Evidence of hypnotic action is lacking.

Polymerization of the lower aliphatic aldehydes occurs readily and, if carried out in the proper manner, results in the formation of trimeric aldehydes (X), which,



(X)

chemically, are not aldehydes but cyclic ethers. Paraldehyde, the trimer of acetaldehyde, has structure (X) in which R is a methyl group. Paraldehyde is one of the older organic synthetic hypnotics. Its actions are similar to those of alcohol, even including habituation and delirium tremens. It is less potent and less toxic than chloral but it also has less tendency to depress the heart and respiration. The main disadvantages of paraldehyde are its irritating effects upon mucous membranes and the persistent fusel-oil odor imparted to the breath. Other trimeric aldehydes are devoid of any useful hypnotic action. Knoefel (9) found the properties of the trimers of formaldehyde, propionaldehyde, *n*-butyraldehyde and isobutyraldehyde to be either too toxic or lacking completely in hypnotic activity.

Acid Amides, Acyl Ureas and Alkyl Ureas.—The hypnotics belonging to these three classes of synthetic organic compounds are suitably discussed as a group since they are closely related in structure and activity, and the same types of structural changes cause similar alterations in physiological behavior. Even the most active members must be classified as weak hypnotics; in fact, it would be more accurate to limit description of their action to the statement that they are sedatives because none of the compounds has any actual value as a hypnotic, whereas several derivatives have proved useful in the treatment of mild nerve disorders such as simple nervous insomnia.

The acid amides, acyl ureas and alkyl ureas of simplest structure, for example, acetamide, acetyl urea and ethyl urea, are very weak depressants, the activity of which can be increased several-fold by the introduction of alkyl and aryl groups as well as of chlorine and bromine atoms. All three types of compounds have been the subjects of systematic studies which have contributed a total knowledge sufficient for one to conclude that, unless some radically new de-

parture from the accepted scheme of structural manipulation is developed, the possibility of finding a truly outstanding hypnotic in this group is slight.

The acid amides of highest activity consist of derivatives of acetamide in which hydrogens of the methyl group have been replaced by two (10) or three alkyl groups or by a combination of two alkyl groups and a chlorine or bromine atom. Some of these compounds are interesting because they possess a combined sedative and analgesic action (10). Other derivatives containing two or three alkyl groups in addition to a methyl radical on the amido nitrogen produce a mild, prolonged period of sedation in experimental animals (11, 12). Examples of the more active acid amides include: diethylallylacetamide (Novonal), α-chlorodiethylacetamide (Declonal), ethylisopropylacetamide (Neodorm) and ethyl 1-methyl butylacetamide (10).

The degree of potency of the acyl ureas (ureides) is increased, in general, by the same types of structural change that affect the acid amides (10, 11, 12). Replacing the urea oxygen by sulfur to give acyl thioureas leads to inactive compounds (13). Certain symmetrical diacyl ureas are comparable in potency to barbital (14) although the duration of action is very short, lasting only one to two minutes when the substance is administered intravenously to experimental animals. The duration of action is more prolonged (15–60 minutes) following intraperitoneal injection and the required dose is also much greater (15). The activity of the diacyl ureas is not increased by the presence of a tertiary alkyl group but the rate of destruction in the body is reduced; hence duration is prolonged. Hypnosis caused by administration of diacyl ureas is accompanied by marked analgesia.

The general type of action to be expected from the most active acyl ureas is exemplified by some of the conditions that respond to treatment with bromodiethylacetyl urea (Carbromal), namely, hysteria, cardiac neuroses with tachycardia, mental disorders with moderate excitement, and insomnia due to various intestinal diseases (16). Bromoisovaleryl urea (Bromural) and allylisopropylacetyl urea (Sedormid) are other examples of useful ureides.

The alkyl ureas were investigated systematically by Buck, Hjort, DeBeer and coworkers (17) and some conclusions reached were: (*a*) molecular weight of alkyl ureas is a determining factor of hypnotic effectiveness; (*b*) among the aliphatic ureas, hypnotic potency decreases in the order monoalkyl, sym.-alkyl methyl, unsym.-dialkyl, sym.-alkyl ethyl and trialkyl ureas; (*c*) molecular weight is not a deciding factor for potency among the aryl and alkylaryl ureas, the position of a substituent group in the benzene ring being more important; (*d*) halogenation (Cl, Br) of alkyl and alkylaryl ureas increases hypnotic potency. The alkyl ureas do not appear to be represented among the sedatives or hypnotics that are in clinical use.

Urethanes.—This term is applied to the class of compounds consisting of esters of carbamic acid (NH_2COOH) or alkyl carbamates and it is frequently used to denote the ethyl ester of the series. Ethyl carbamate has long been known to possess somnifacient properties although, considering its effectiveness and rapidity of action in experimental animals, its action in man has been disappointing. Ethyl carbamate was formerly employed to some extent as a sedative and hypnotic in the clinic but its use has been discontinued because numerous other more desirable compounds are available.

Measures effective in increasing the potency of the acid amides, acyl ureas and alkyl ureas will also improve the hypnotic properties of the urethanes, the esters of certain branched chain and chlorinated alcohols being the most active. Although several such derivatives have been placed on the market, for example, 1-methylbutyl carbamate (Hedonal), tertiary amyl carbamate (Aponal), β-trichloroethyl carbamate (Voluntal) and α,α'-dichloroisopropyl carbamate (Aleudrin), none of them has shown any great promise because the increase in activity is accompanied by a corresponding increase in toxicity.

In general, the urethanes are not sufficiently active hypnotics to warrant their use as such in medicine and there is little reason to believe that additional investigations will change this situation if they are patterned after the present-day methods for increasing effectiveness. The chances of developing a derivative of carbamic acid into a useful hypnotic are also limited by the fact that the introduction of radicals other than alkyl or acyl usually causes a complete change in physiological activity. The local anesthetic properties of the phenyl urethanes serve as an outstanding example of this behavior.

Barbiturates.—Any discussion of the hypnotics must necessarily include a special consideration of barbituric acid derivatives because of the wide usage of, and many advantages possessed by, these compounds in comparison with other drugs having a similar action. Full realization of the unique physiological behavior of the barbiturates

was initiated by the report of Shonle and Moment (18) who were the first to investigate the activity of the unsymmetrical 5,5′-dialkylbarbituric acids. Subsequent studies have resulted in the preparation and testing of a total of many hundreds of barbiturates and it is therefore possible today to summarize rather completely the relationships between chemical structure and physiological activity.

Barbituric acid and its derivatives (VII) are cyclic ureides resulting from the condensation of a malonic acid derivative with urea or a substituted urea. In order to obtain compounds with hypnotic properties it is essential that both the 5- and 5′-positions be occupied by groups belonging, in general, to the classes consisting of alkyl, alkenyl, aryl or cycloalkyl radicals. The most active compounds are those which contain two such groups, different in size, structure or configuration. The hypnotic action of the 5,5′-substituted barbituric acids is greatly influenced by the presence of substituents on the urea nitrogen atoms (1,3-positions) and by the substitution of the urea oxygen by sulfur.

In the most active barbiturates the sum of the carbon atoms in the 5,5′-positions is seven or eight (18). This combination often consists of an ethyl group and a normal or secondary alkyl group, the latter being more effective in increasing activity (2). The 5,5′-dialkyl-N-methylbarbituric acids display an intense hypnotic action (19), the onset of which may be so rapid that the test animal is asleep before the full dose has been administered.

The newest advancement in barbiturate therapy consists in the intravenous administration of certain thiobarbiturates for producing general anesthesia. Although Fischer and von Mering (20) considered the thiobarbiturates to be too toxic for clinical use, their conclusion must be discarded in view of more recent studies (19, 21, 22) which have demonstrated the utility of such compounds as 5-ethyl-5′-(1-methylbutyl)-thiobarbituric acid (Pentothal) and 5-ethyl-5′-isoamylthiobarbituric acid (Thioethamyl). When administered according to rigidly prescribed technique, the thiobarbiturates produce an intense anesthetic reaction of short duration. A condition of general anesthesia is obtained in less than one minute from the time administration is started and the duration of action of the initial dose is sufficiently short that additional quantities must be given if the operation occupies more than fifteen to twenty minutes (23).

All of the barbiturates produce, in general, the same reactions although they differ widely in speed of onset of action and duration of hypnotic effects, the latter factor being governed by the rate of destruction or elimination in the body or both. Depression of the central nervous system may be obtained in any degree from slight sedation to general anesthesia by choosing the proper member of the series and administering the designated dose. Barbiturates are employed as sedatives, hypnotics, anticonvulsants, basal anesthetics and general anesthetic agents. While they have no analgesic properties, simultaneous administration with such compounds as salicylates, antipyrine and acetophenetidine gives rise to a potentiated analgesic reaction. Phenobarbital is distinguished from all the other barbiturates by its selective action on the motor cortex which makes it a valuable agent for the control of epilepsy. Thiobarbiturates are limited in use to production of general anesthesia by intravenous injection.

Hypnotic doses of the barbiturates seldom cause serious secondary reactions although a period of pre-excitement, even to the extent of delirium, is sometimes experienced. The respiration and blood pressure are only slightly depressed, probably due mainly to the sleep produced. Intravenous injection may cause a sharp but transitory fall in blood pressure. Doses larger than those required for hypnosis act as direct depressants on the medullary center and the central vasomotor center resulting in a decrease in both rate and depth of respiration (24), vasodilatation and hypotension.

The barbiturates are either eliminated largely unchanged by the kidneys or destroyed in the liver. In many instances both mechanisms are active in removing a specific barbiturate from the body. According to Reinert (25) both the duration of action and the toxicity are governed to a large extent by the ability of the liver to destroy and of the kidneys to excrete the compounds. The shortest-acting barbiturates are probably destroyed completely by the liver while those with a prolonged duration of action are mainly excreted. In any case the rate of removal is sufficiently rapid that cumulative toxicity is not a factor.

The possibilities of barbiturate addiction have received much prominence in recent years, particularly since the inauguration of laws governing the sales of barbituric acid compounds in many of the states. Addiction probably does not occur but habituation is rather common. It is important to emphasize the distinction because the symptoms of withdrawal of the barbiturates are limited to a small percentage of patients who

experience mild craving and psychic disturbances (26), whereas withdrawal following true drug addiction leads to serious and practically universal symptoms. It is probable that no significant degree of tolerance is developed (27) and that there is no appreciable change in the toxic dose after continued administration. These same conclusions were reached by Swanson and coworkers (28) after giving repeated doses of amytal to dogs and monkeys.

In view of the large number of compounds now on the market, at first glance it might appear nearly impossible for the physician to make an intelligent and proper choice of the barbiturate best suited for a specific patient. However, the situation is considerably simplified by the fact that many of the commercial preparations are quite similar in activity, toxicity, duration of action, and therapeutic uses despite numerous claims of individual superiority. The physician has only to familiarize himself with the properties of some six or eight different barbiturates in order to take advantage of the important differences in duration of action and specific uses of the entire series. Despite some evidence to the contrary, the margin of safety (*i. e.*, the ratio between the minimum effective dose and the minimum toxic dose) is not an important factor of difference among the barbiturates. In the words of the Council on Pharmacy and Chemistry of the American Medical Association "... there is no satisfactory evidence that the margin between the therapeutic and toxic doses of these derivatives is significantly wider than in the case of barbital itself" (29). An approximate comparison of duration of action and hypnotic dose of some of the more important barbiturates appears in Table I.

Hydantoins.—The similarity between the barbiturates (VII) and the hydantoins (VIII) becomes apparent upon comparing their respective structures. It will be noted that the hydantoin nucleus contains one carbonyl group less than barbituric acid; otherwise the structures are identical. The analogy between the two types of compounds also extends to their chemical reactivity in that various alkyl, aryl and other groups may be introduced into the 5-positions of hydantoin. These relationships were probably factors in stimulating the original investigations of hydantoins as hypnotics.

TABLE I.—BARBITURIC ACID DERIVATIVES

Trade Name	R and R′	Duration of Action[a]	Hypnotic Dose, Gm
Alurate	Allyl isopropyl	Long	0.065–0.13
Amytal	Ethyl isoamyl	Moderate	0.1 –0.3
Barbital (Veronal)	Diethyl	Long	0.3 –0.5
Cyclopal	Allyl Δ^2-cyclopentenyl	Moderate	0.12 –0.25
Delvinal	Ethyl (1-methylbutenyl)	Moderate	0.1 –0.2
Dial	Diallyl	Long	0.1 –0.3
Evipal	Methyl cyclohexenyl 1-methyl	Short	0.25 –0.4
Ipral	Ethyl isopropyl	Long	0.12 –0.25
Neona	Ethyl *n*-butyl	Long	0.05 –0.1
Orta	Ethyl *n*-hexyl	Moderate	0.2 –0.4
Pentobarbital	Ethyl (1-methylbutyl)	Moderate	0.1 –0.2
Pentothal	Ethyl (1-methylbutyl) 2-thio-	Very short	Anesthetic only
Phenobarbital (Luminal)	Ethyl phenyl	Long	0.1 –0.2
Phtaodorn	Ethyl cyclohexenyl	Moderate	0.1 –0.2
Sandoptal	Allyl isobutyl	Moderate	0.2 –0.4
Seconal	Allyl (1-methylbutyl)	Short	0.1 –0.2
Sigmodal	(β-Bromallyl) (1-methylbutyl)	Moderate	0.1 –0.2
Thioethamyl	Ethyl isoamyl 2-thio-	Very short	Anesthetic only

[a] Terms used to describe duration of action indicate periods of hypnosis lasting approximately as follows: long—6 to 12 hr.; moderate—3 to 6 hr.; short—½ to 3 hr.

The first of the hydantoins to be reported as having somnifacient properties was the 5-ethyl-5′-phenyl derivative (30), an analogue of phenobarbital which became known as Nirvanol. This compound soon proved to be a poor hypnotic but it did find limited use in the treatment of chorea in children. It later became apparent that its action is not specific but depends upon development of toxic effects. No benefits are derived from the drug unless enough is administered to cause high fever, blood changes and skin rash. Such behavior is sufficient to exclude its use as a medicinal agent. Other 5-alkyl-5′-phenyl hydantoins which have been prepared and tested (31) were found to be similar to Nirvanol or completely inactive. Further investigations (32, 33) which included studies of various phenylalkyl and tetrahydropyranyl hydantoins led to the conclusion that hydantoins in general are poor hypnotics although certain derivatives

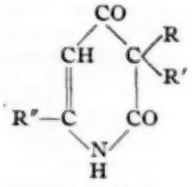
show interesting anticonvulsant properties.

The only hydantoin with useful clinical properties is the 5,5′-diphenyl derivative (Dilantin) which was first investigated by Putnam and Merritt (34) and has since been proved to be a valuable agent in the treatment of epilepsy. The drug is effective in completely relieving or greatly improving the condition of over 80% of the cases (35) and it is effective against the *grand mal* type of seizure where bromides or phenobarbital are comparatively useless. Dilantin has an additional advantage over phenobarbital in that it lacks the depressant effects of the latter compound while it is just as active as an anticonvulsant.

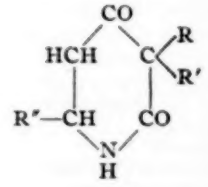
Disulfones.—The disulfones (IX) are important only from a historical standpoint because their use has been practically discontinued. Sulfonmethane (diethylsulfondimethylmethane) and two closely related derivatives obtained by replacing first one and then both methyl groups by ethyl radicals were introduced as hypnotics by Bauman and Kast in 1888 under the names of Sulfonal, Trional and Tetronal, respectively. These compounds are powerful hypnotics with almost no analgesic properties. For some years they found considerable use, but with the development of new hypnotics, particularly the barbiturates, and a fuller knowledge of the toxicity of the sulfonmethanes, they have fallen into disuse. All three are very poorly soluble in water and for this reason they require two to five hours for their effects to take place. Once absorbed they are excreted slowly while after-depression is pronounced and universal. Because of their low rates of absorption and excretion, continued administration results in cumulative toxic reactions. Considering the large selection of other less toxic hypnotics available today, there is little reason for further use of the sulfonmethanes.

Unclassified Hypnotics.—There are a large number of organic compounds belonging to chemical classes other than those discussed above which have been described as having soporific properties but which, so far as can be ascertained, possess insufficient activity to be of medicinal value. Certain similarities in structure exist between these compounds and others of established therapeutic action which are interesting and important to the extent that they add to our knowledge of the relationships between chemical structure and hypnotic activity. It is impossible to include all such compounds in this discussion but brief mention of a few outstanding examples seems desirable.

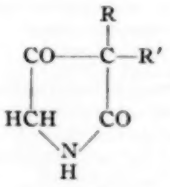
The group $RR'=\overset{|}{C}-CONH-$ is present in several types of compounds, many derivatives of which are active hypnotics. At least five other classes having this radical in common have been shown to include members which possess hypnotic properties, namely, the 2,4-diketo-3,3-dialkyl derivatives of tetrahydropyridine (XI) (37), piperidine (XII) (38) and pyrrolidine (XIII) (39), the 5,5-dialkyl-2,4-oxazolidinediones (XIV) (40) and the 5,5-dialkyl-2,4-thiazolidones (XV) (41).



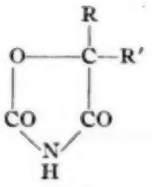
(XI)



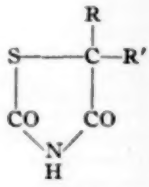
(XII)



(XIII)



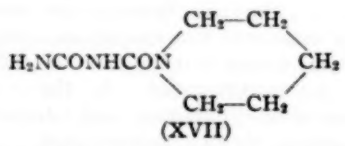
(XIV)



(XV)

In pharmacological tests some of the dialkylacetyl biurets (XVI) and thiobiurets compare favorably with sodium barbital in hypnotic effectiveness (42). Activity is increased by the presence of branched chains in the acyl radical and by alkylation in the 5-position of the biuret structure. The dialkylacetyl biurets have a surprisingly low toxicity while 5,5-pentamethylenebiuret (XVII), as well as 1,1-pentamethylene-5,5-pentamethylenebiuret, is less toxic than the sodium salts of barbital, pentobarbital or diphenylhydantoin (43). The last two compounds are said to resemble pentobarbital in their hypnotic effects.

RCONHCONHCONHR′
(1) (2) (3) (4) (5)
(XVI)



(XVII)

It is gratifying to note that progress in the field of hypnotics has reached a point where reasonably satisfactory agents are available for the symptomatic treatment of the majority of conditions and diseases requiring

sedation and hypnosis for their alleviation and cure. Although none of these drugs will fulfill all the requirements for the so-called ideal hypnotic, we are approaching sufficiently close to this ultimate goal that it is no longer necessary to use hypnotics which will bring uncertain, unpleasant or dangerous results. Advancement has been impeded by lack of a fuller understanding of the mechanisms by which these drugs act and it is perhaps this phase of the subject that offers the most promising basis for outstanding advancements in the future. The desire to better the properties of present-day hypnotics constantly stimulates investigation and, judging from the progress made so far, there is every reason to expect still further improvements.

REFERENCES

(1) Sollmann, T., "A Manual of Pharmacology," ed. 6, W. B. Saunders Co., Philadelphia, 1943, (*a*) p. 690, (*b*) p. 701, (*c*) p. 771.

(2) Shonle, H. A., *J. Ind. Eng. Chem.*, 23 (1931), 1104.

(3) Richardson, B. W., *Med. Times and Gaz.*, 18 (1869), 703.

(4) Jenkins, G. L., and Hartung, W. H., "The Chemistry of Organic Medicinal Products," ed. 2, John Wiley and Sons, New York, 1943, p. 70.

(5) Molitor, H., and Robinson, H., *Anesthesia and Analgesia*, 17 (1938), 258.

(6) Burtner, R. R., and Lehmann, G., *J. Pharmacol.*, 63 (1938), 183.

(7) Lehmann, G., and Knoefel, P. K., *Ibid.*, 63 (1938), 453.

(8) Belfrage, D., *Lancet*, 235 (1938), 1290.

(9) Knoefel, P. K., *J. Pharmacol.*, 48 (1933), 488.

(10) Volwiler, E. H., and Tabern, D. L., *J. Am. Chem. Soc.*, 58 (1936), 1352.

(11) Blicke, F. F., and Centolella, A. P., *Ibid.*, 60 (1938), 2924.

(12) Nelson, J. W., Lyster, S. C., and Cartland, G. F., THIS JOURNAL, 30 (1941), 180.

(13) Moore, M. L., and Crossley, F. S., *J. Am. Chem. Soc.*, 62 (1940), 3273.

(14) Stoughton, R. W., *J. Org. Chem.*, 2 (1938), 514.

(15) Stoughton, R. W., Dickison, H. L., and Fitzhugh, O. G., *J. Am. Chem. Soc.*, 61 (1939), 408.

(16) "New and Nonofficial Remedies," American Medical Association, 1943, p. 470.

(17) DeBeer, E. J., Buck, J. S., Hjort, A. M., *et al.*, *J. Pharmacol.*, 52 (1934), 216; 54 (1935), 188; 55 (1935), 157; 60 (1937), 369; 61 (1937), 175.

(18) Shonle, H. A., and Moment, A., *J. Am. Chem. Soc.*, 45 (1923), 243.

(19) Tabern, D. L., and Volwiler, E. H., *Ibid.*, 58 (1936), 1355.

(20) Fischer, E., and von Mering, *Therapie der Gegenwart*, 44 (1903), 97.

(21) Tabern, D. L., and Volwiler, E. H., *J. Am. Chem. Soc.*, 57 (1935), 1961.

(22) Miller, E., Munch, J. C., and Crossley, F. S., *Science*, 81 (1935), 615; Miller, E., Munch, J. C., Crossley, F. S. and Hartung, W. H., *J. Am. Chem. Soc.*, 58 (1936), 1090.

(23) Lundy, J. S., *Proc. Staff Meetings Mayo Clinic*, 10 (1935), 257.

(24) Page, I. H., and Coryllos, P., *J. Pharmacol.*, 27 (1926), 189.

(25) Reinert, M., *Arch. exptl. Path. Pharmakol.*, 130 (1928), 49.

(26) Hambourger, W. E., *J. Am. Med. Assoc.*, 114 (1940), 2015.

(27) Stanton, E. J., *J. Pharmacol.*, 57 (1936), 245.

(28) Swanson, E. E., Weaver, M. M., and Chen, K. K., *Am. J. Med. Sci.*, 193 (1937), 246.

(29) "New and Nonofficial Remedies," American Medical Association, 1943, p. 478.

(30) Wernecke, *Deut. med. Wochschr.*, 42 (1916), 1193.

(31) Thompson, T. J., Bedell, H. L., and Buffett, G. M., *J. Am. Chem. Soc.*, 47 (1925), 874.

(32) Herbst, R. M., and Johnson, T. B., *Ibid.* 54 (1932) 2463.

(33) Rigler, N. E., and Henze, H. R., *Ibid.*, 58 (1936), 474; Whitney, W. B., and Henze, H. R., *Ibid.*, 60 (1938), 1148; Henze, H. R., and Long, L. M., *Ibid.*, 63 (1941), 1936; Henze, H. R., and McKee, R. L., *Ibid.*, 64 (1942), 1672.

(34) Putnam, T. J., and Merritt, H. H., *Science*, 85 (1937), 525; *Arch. Neurol. Psychiat.*, 39 (1938), 1003.

(35) Putnam, T. J., and Merritt, H. H., *J. Am. Med. Assoc.*, 111 (1938), 1068; *Arch. Neurol. Psychiat.*, 42 (1939), 1053; *Am. J. Psychiat.*, 96 (1940), 711.

(36) Robinson, L. J., and Osgood, R., *J. Am. Med. Assoc.*, 114 (1940), 1334.

(37) Schnider, O., Festscrift, Emil C. Barell, (1936) 195; U. S. Patents 2,090,068 and 2,090,237, August 17, 1937.

(38) U. S. Patent 2,151,047, March 21, 1939.

(39) German Patent 695,330, July 25, 1940.

(40) Stoughton, R. W., and Baxter, J. H., *J. Pharmacol.*, 73 (1941), 45.

(41) Doran, W. J., and Shonle, H. A., *J. Org. Chem.*, 3 (1939), 193.

(42) Hill, A. J., and Degnan, W. M., *J. Am. Chem. Soc.*, 62 (1940), 1595.

(43) Anderson, H. H., Ch'eng, C. H., P'an, S.-Y., Sah, P. P. T., and Lu, C.-S., *Science*, 95 (1942), 254.

Oils of Cinnamon—Properties and Assays*,†

By Roy C. Darlington‡ and B. V. Christensen§

In a previous paper (1) the results of several methods of assay for total aldehydes of oil of cassia were reported. The percentage yield of several extracted cinnamon oils and the physical constants of these oils as well as those of several commercial cassia oils were also given. The purpose of this paper is to report the results of the continued investigation of the physical properties and the assay of oil of cassia.

This study of cassia oil was undertaken in an attempt to solve the following problems concerning authentic oil of cassia.

1. What should be the percentage by volume of total aldehydes?
2. What should be the percentage by weight of *ortho*-methoxy cinnamic aldehyde?
3. What is a reliable method for the determination of total aldehydes?
4. What is a reliable method for the determination of *ortho*-methoxy cinnamic aldehyde?
5. What is the range of refractive index of an authentic oil of cassia?

Attempts to obtain the leaves and twigs of *Cinnamomum cassia* being unsuccessful, the cassia bark was used as a source of the oil. In addition to two extracted cassia oils, several commercial oils, both redistilled and technical, were used in this study.

EXPERIMENTAL

The cassia oil was extracted by dry steam distillation in the Lloyd extractor. Two fractions were obtained, one which separated in the original distillation, and another which was obtained by cohobation. The oils were dried over anhydrous sodium sulfate and stored in amber-colored bottles. The yield was 1.02%. The physical constants of the extracted and commercial oils are given in Table I.

The specific gravity of the uncohobated-extracted oil was below the specification in the U. S. P., while those of the cohobated and commercial oils were within the limits of the U. S. P. requirement. The refractive indices of the cohobated and uncohobated-extracted oils were not within the limits of the U. S. P. requirement, that of the former being higher and that of the latter being lower. The refractive index of the commercial oil was within the limits of the U. S. P. requirement.

Total Aldehyde Assay.—It was previously stated that the U. S. P. XII method of assay for total aldehydes in oil of cassia was found to be unsatisfactory by the writer. It was further stated that the sulfite method of assay was the most satisfactory method of those studied at that stage of this investigation. However, this method suffers from the disadvantage that there is no indication of the end point of the reaction. Since then another method has been tried and found to be satisfactory in every way. The procedure in detail follows.

Sulfite-Bisulfite Method of Assay for Total Aldehydes.—Procedure: Mix 15 cc. of 25% sodium sulfite and 35 cc. of 25% sodium bisulfite in a 100-ml. cassia flask, insert thermometer and heat in a water bath to a temperature of 75° C. Remove from the water bath, add 3 drops of phenolphthalein T. S., 10 cc. of cassia oil, insert thermometer and shake flask gently from side to side. Maintain neutrality by adding 25% sodium bisulfite from a pipette. Allow sodium bisulfite to run down neck of rotating flask. This serves to remove any particles adhering to the neck of the flask. Continue shaking of flask until the temperature ceases to rise and returns to 75° C. Remove thermometer, stopper flask, and when the mixture has cooled to room temperature and when the liquids have separated completely, add sufficient 25% sodium sulfite, which has been neutralized to 3 drops of phenolphthalein T. S. with 25% sodium bisulfite, to raise the lower limit of the oily layer within the graduated portion of the neck.

The result of the assays of several oils by this method are given in Table II.

Assay for ortho-Methoxy Cinnamic Aldehyde.—The method used in the determination of the *ortho*-methoxy cinnamic aldehyde content of the various cassia oils was that of Viebock and Schwappach as modified by Clark (2). In Table III, the results of the assays are given along with the total aldehyde content of the various oils.

TABLE I

Oil	Index of Refraction at 20° C.[a] Observed	Corrected	Specific Gravity 25° C.
Extracted-cohobated	1.6158	1.6148	1.0460
Extracted-uncohobated	1.6020	1.6010	1.0329
Commercial-technical	1.6049	1.6039	1.0588

[a] Abbe refractometer correction, −0.001°.

DISCUSSION

Sulfite-Bisulfite Method for Total Aldehydes.—In the development of this method of assay, various preparations of the sulfite-bisulfite mixture were used in the assay of cassia oils containing from 82 per cent to 95 per cent total aldehydes. It was found that the mixture, 15 cc. sodium sulfite —35 cc. sodium bisulfite, was most satisfactory for assaying oils that have an aldehyde content within the range of U. S. P. specifications. To overcome the objection

* Received Oct. 15, 1943, from the College of Pharmacy, Ohio State University, Columbus, Ohio.

† Abstract of a thesis presented to the Graduate School, Ohio State University, in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

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to the bisulfite method mentioned above, it was necessary to develop some means of indicating the end point of this assay.

The aldehyde-bisulfite reaction is exothermic and hence there will be a rise in temperature as the reaction proceeds. It is also known that an increase in temperature brings about a corresponding increase in the rate of such a reaction. Therefore, it was deemed advisable to increase the temperature of the reaction mixture. Various temperatures were tried and 75° C. was found to be the optimum temperature for the reaction. The end of the reaction is indicated by the cessation of the rise in temperature of the reaction mixture. Shaking of the mixture is continued until the temperature returns to 75° C. merely as an added assurance that the reaction is completed. The amount of 25 per cent sodium bisulfite needed for neutralization in this assay will vary with the total aldehyde content of the oil under test, a higher grade oil obviously requiring more than a lower grade oil. The writer has found this amount to vary from 18 cc. with an oil of 82 per cent total aldehydes to 28 cc. with an oil of 95 per cent total aldehydes. This method is recommended because it is simple, rapid and sufficiently accurate.

Assay for ortho-Methoxy Cinnamic Aldehyde.—In applying the methoxy test to oil of cassia, certain precautions must be observed (3). The oil must be free from water-soluble alcohols, esters and ethers. The presence of these substances may be detected by washing the oil with water, drying and checking the refractive index, which should be unchanged. Ethers and esters which are insoluble in water lower the aldehyde content and will be found in the non-aldehydic portion of the oil, which will show an abnormally high methyl value. Hence it is seen that the methoxy test alone is of little value but must be considered in conjunction with the aldehyde determination as well as the physical constants.

Several difficulties were encountered in the application of this test to oil of cassia. The first, that of bumping, was overcome by changing the type of apparatus. The apparatus[1] which eliminated the bumping is designed so that the stream of carbon dioxide bubbles through the reaction mixture before sweeping the apparatus. This promotes smooth boiling in addition to sweeping the volatile iodide into the receiving flasks. Another important change is the source of heat. While the reaction flask of the apparatus previously used was heated with a microburner, that of the latter is heated by the condensate of a liquid which boils at a temperature slightly higher than that of the reaction mixture. In these determinations cyclohexanol was used.

Because the methoxyl determination has not been extensively used in the assay of oil of cassia, it was thought advisable to check thoroughly the reliability of this method in regard to oil of cassia.

In the usual methoxyl determinations of pure chemicals a blank is run on the reagents to be used and this is subtracted from the first determination. Then at least two more determinations may be made on the same charge of hydriodic acid and phenol. This was not found to be the case, however, with oil of cassia. An increase in the blank obtained was noted over a period of three hours. Furthermore, forty-five minutes to one hour was found to be the optimum time for running a determination, as extension of the time gave high results. Another factor noted was that the reagents in the reaction flask must be changed after each determination as repeated runs on the same reagents gave high results, probably due to degradation products. If the above precautions were observed, results could be duplicated and the analyses checked with those of the analysts of volatile oil companies.

Dodge, from the results of his work on methoxyl determinations on oils of cassia, concluded that an authentic oil of cassia probably contained from 10–14% *ortho*-methoxy cinnamic aldehyde. As may be seen in Table III, the commercial oils analyzed are in perfect agreement with his postulation. The bark oils, however, are seen to fall far below this specification.

TABLE II

Oil Used	Per Cent of Total Aldehydes
Commercial (U. S. P.)	92
Commercial (U. S. P.)	84
Commercial (U. S. P.)	86
Commercial (technical)	82
Commercial (technical)	86.5
Extracted Cassia (cohobated)	95
Extracted Cassia (cohobated)	92
Extracted Cassia (uncohobated)	86

TABLE III

Oil Used	Total Aldehyde, %	*ortho*-Methoxy Cinnamic Aldehyde, %
Commercial (U. S. P.)	92	10.98
Commercial (U. S. P.)	84	11.22
Commercial (U. S. P.)	86	10.43
Commercial (technical)	82	12.61
Commercial (technical)	86.5	11.37
Extracted Cassia (cohobated)	95	3.21
Extracted Cassia (cohobated)	92	4.44
Extracted Cassia (uncohobated)	86	4.40

From the results obtained in this laboratory it can only be concluded that either the oil of the leaves and twigs is of a different constitution than that of the bark or that most of the methoxyl content of the commercial oils is a carryover from previous adulteration. The oils extracted in this laboratory are, without doubt, high-quality oils with a high total aldehyde content yet low *ortho*-methoxy cinnamic aldehyde content. Hence it is believed that the percentage of total aldehydes is more of a criterion of the quality of the oil than is the methoxy value. This is not meant as a denial of the value of the methoxy test as an aid in the detection of the adulteration of cassia oil.

SUMMARY

1. The several problems, whose solutions were attempted in this investigation, are given.

[1] Modifications designed by Miss Jean Anderson, Department of Chemistry, Ohio State University.

2. The physical constants of extracted cassia oils and commercial cassia oils were determined.

3. A new method of assay for total aldehydes in oil of cassia is given and discussed.

4. The methoxy assay as applied to oil of cassia is discussed.

CONCLUSIONS

1. The total aldehyde content of oil of cassia bark may range from 86 per cent in an uncohobated oil to 95 per cent in a cohobated oil. Cohobation produces a higher grade oil in terms of aldehyde content.

2. The sulfite-bisulfite method is a reliable method of determining the total aldehyde content of oil of cassia.

3. The percentage by weight of *ortho*-methoxy cinnamic aldehyde in the bark oil is very low. What the percentage in an authentic oil should be must be determined by obtaining an unadulterated oil from the leaves and twigs of *C. cassia*.

4. The method for methoxy determination as outlined by the writer is reliable for the determination of *ortho*-methoxy cinnamic aldehyde in oil of cassia.

5. The refractive index of one cohobated extracted oil exceeded the upper limit of the U. S. P. specification, while that of the uncohobated oil was below the lower limit of the U. S. P. specification.

REFERENCES

(1) Darlington, R. C., and Christensen, B. V., THIS JOURNAL, 32 (1942), 118.

(2) Clark, E. P., *J. Assoc. Official Agr. Chem.*, 15 (1932), 136.

(3) Dodge, F. D., *Am. Perfumer*, March (1939).

Solanum Triflorum Nutt. I. A Preliminary Investigation*,†

By Leo A. Sciuchetti,‡ R. L. McMurray§ and Coy W. Waller‖

Solanum triflorum Nutt., commonly called Cut-leaved Nightshade, Spreading Nightshade, Three-flowered Nightshade and Wild Tomato, is a native solanaceous plant of the Great Plains region of the United States and Canada. Its habitat extends westward from the Great Plains to the coast line of Washington and Oregon. It is a common weed in the Palouse district of southeastern Washington. Nuttall (1) discovered the plant in 1810 near the present site of Mandan, N. D. and was the first to publish its description.

The plant is an annual and grows from 1 to 7 dm. high. It has a tough fibrous root and exceedingly branched hispidulous stems. Adventitious roots are often found on the reclining stems. The deeply pinnatifid leaves are usually nine-lobed, deltoid to lanceolate. The petioles are 2 to 26 mm. long with blades 1 to 4 cm. in length. Opposite the leaves are lateral peduncles, 4 to 22 mm. long, usually with three pedicels bearing small, white, revolute flowers. The flowers ordinarily bloom from June through September. The campanulate calyx and corolla are somewhat pilosulous. The greenish white to dark green glabrous berries, 6 to 18 mm. in diameter, appear as minute green tomatoes. Depending on the maturity of the plant each berry contains from 20 to 90 pale, flattened, obliquely ovate seeds. Further description, geographical distribution and ecology of the plant can be obtained in almost any book on the flora of the Great Plains or Rocky Mountain region.

Among the more than eighteen hundred species of the Solanaceae are found some of our important alkaloidal drugs as well as important farm crops. The known medicinals belonging to the genus *Solanum* include *S. carolinense*, *S. dulcamara*, *S. arrabenta*, *S. mammosum*, *S. oleraceae* and *S. Pseudo-capsicum*. Many other species of Solanums are known to be toxic and are included among our poisonous plants.

Solanum triflorum is the subject of controversy as to its poisonous aspects. Chesnut (2), Pammel (3), Fyles (4) and Muenscher (5) list the plant, especially the fruit, as being poisonous. Other authorities do

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Presented to the Scientific Section, A. PH. A., Columbus meeting, 1943.

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not include it in their listings. It has been rumored, probably due to its possible toxicity, that the fruit of the plant has been a very serious problem to canners, seed packers and farmers who use peas as a source of forage. Chestnut (2), who believed that the poisonous constituent is some compound of solanine, presented no proof of its identity but showed by experiments that berries from Nebraska were poisonous to guinea pigs and rabbits. To discount these reports there is evidence in the literature which states that the berries are used in jams and pies (6).

The available literature disclosed no chemical investigation of the plant. The only indication of its constituents was Chestnut's statement (2) that he believed its poisonous constituent to be a solanine-like compound. The importance of this family of plants justifies the investigation of any member of the family. The object of the investigation was to supply chemical information regarding the constituents and to find possible medicinal application of the plant.

EXPERIMENTAL

Identification.—Before being gathered in large quantities, plants were identified by the aid of specimens studied in the herbarium of the State College of Washington. Afterward the plants were identified botanically from St. John's book (7).

Collection of Material.—The bulk of the material, 197.7 Kg. of green plants, was gathered in late September at a point twenty miles southwest of Colfax, Wash., on U. S. Highway 295. The plants were immediately washed to remove dirt, sand, silica and adhering particles.

After drying at room temperature the plants were segregated into four portions—leaves and flowering tops, stems, roots and fruit (berries). The fruit was further dried on a specially arranged apparatus at a temperature of 30° to 38° C. After complete drying each portion was ground to the desired fineness by means of a Wiley mill. The ground and dried material weighed approximately 31.8 Kg., representing 16.1% of green weight. The ratio of each part by weight to the total after drying and grinding amounted to:

Leaves and flowering tops	40.8%
Fruit	36.8%
Stems	20.6%
Roots	1.8%

Ash Analysis.—The total ash and acid-insoluble ash were obtained from representative samples of about 3 Gm. each, according to U. S. P. XI methods (8). The acid-soluble ash was obtained by difference. The alkalinity of the water-soluble and water-insoluble ash and the acid-soluble and acid-insoluble ash was determined by methods from Woodman (9). The values are in terms of per cent of air-dried material. The alkalinity is expressed in terms of cubic centimeters of $N/10$ acid per gram of air-dried sample. The average of three determinations on each portion is tabulated in Table I.

Moisture Determination.—Moisture was determined for the air-dried samples by the official toluene method as described by the U. S. P. XI (10). The following average results expressed in per cent were obtained:

Leaves and flowering tops	8.12
Fruit	9.63
Stems	6.90
Roots	6.46

Fig. 1.—*Solanum triflorum.*

TABLE I.—ASH ANALYSIS OF *Solanum Triflorum*

Sample	Leaves and Flowering Tops	Fruit	Stems	Roots
Total ash	18.06	8.87	12.92	11.00
Water-insoluble ash	10.22	2.79	3.50	4.93
Water-soluble ash	7.84	6.08	9.42	6.07
Acid-insoluble ash	2.44	0.76	0.48	1.56
Acid-soluble ash	15.62	8.08	12.44	9.44
Alkalinity of water-soluble ash	6.38	5.43	9.03	5.96
Alkalinity of water-insoluble ash	11.20	3.61	5.92	6.39

Dragendorff Extraction.—Twenty-three-gram samples of the leaves and flowering tops and 30-Gm. samples of the fruit were extracted with various solvents according to the method of Dragendorff (11), but modified briefly. The petroleum-ether and anhydrous-ether extractions were continued for forty-two hours, and the alcoholic extraction over one hundred hours. A Soxhlet extractor was used and each solvent was tested for complete extraction. The volatile solvents were allowed to evaporate spontaneously and later dried to constant weight at 100° C. The petroleum-ether extract from the fruit was a dark green oil. That from the leaves and tops consisted of a dark brownish green waxy solid. All anhydrous-ether extracts were green solids. Simple tests indicated the possible presence of saponins from the alcoholic extract of the fruit. The average results stated in per cent of the air-dried samples were:

	Leaves and Tops	Fruit
Petroleum ether	3.06	8.34
Anhydrous ether	1.06	1.56
Alcohol	15.25	19.73

Crude Fiber, Volatile Ether-Soluble Extractive and

Nonvolatile Ether-Soluble Extractive.—Two grams of the previously prepared drug was treated as directed by the official U. S. P. XI methods (12) and the crude fiber, volatile ether-soluble extractive and nonvolatile ether-soluble extractive were determined. The average results stated in per cent of air-dried samples were:

	Leaves and Tops	Fruit
Crude fiber	10.92	20.29
Volatile ether-soluble extractive	0.64	0.82
Nonvolatile ether-soluble extractive	3.88	8.64

Starch and Sugar.—Simple microchemical tests on sections of the fruit showed the presence of starch. Caramelization during the grinding of the fruit indicated the possibility of sugars.

Fixed Oil Obtained by Petroleum-Ether Extraction.—6.36 Kg. of air-dried fruit was extracted directly in a specially constructed Soxhlet extraction apparatus in order to make a more complete examination of the oil. The percolates were gathered at various times during the extraction which was continued intermittently over a period of five days. Each portion was dried with anhydrous sodium sulfate, stoppered and allowed to stand overnight, filtered, and the solvent was removed by distillation. The remaining traces of solvent were removed by spontaneous evaporation, then by heat over a water bath, and finally by vacuum. The portions were combined and 480 Gm. or 7.55% of petroleum-ether extract was obtained. The fixed oil thus extracted had a dark green color indicating the presence of a large amount of chlorophyll. A characteristic odor resembling crushed tomato leaves and a greasy repellent taste were other properties of the oil. The following constants obtained by official U. S. P. XI methods were determined (13):

Specific gravity, 25° C	0.9229
Refractive index, 25° C	1.4761
Optical rotation[a]	None
Saponification number	176.7
Acid value	20.1
Ester value	156.6
Iodine value	122.9
Unsaponifiable matter	5.02%

[a] A chloroform solution of the oil decolorized by norite was used in this determination.

Alkaloidal Assays.—Two 25-Gm. samples each of the leaves and tops and fruit were assayed for total Belladonna alkaloids according to U. S. P. XI methods (14). Negative results were obtained from the fruit, whereas the leaves and tops gave slightly positive results corresponding to 0.0081 Gm. of total Belladonna alkaloids representing 0.0324%.

SUMMARY AND CONCLUSIONS

A brief review of the literature and a description of *Solanum triflorum* have been presented. A preliminary investigation has been made. Data for the leaves and flowering tops, fruit, stems and roots are reported for ash and moisture. The following results for the leaves and flowering tops and fruit are reported: Dragendorff extraction, crude fiber, volatile ether-soluble extractive and nonvolatile ether-soluble extractive. The high crude fiber content might not justify the use of the fruit as a food, but the known occurrence of starch and possible presence of sugars justify the use of the berries in making jellies and pies.

The physical and chemical constants of the fixed oil obtained from the petroleum-ether extraction are reported. This oil may be classified as a semidrying oil with a relatively large proportion of low molecular weight fatty acids.

The 5.02 per cent of unsaponifiable matter which was found in the petroleum-ether extract is considerably larger than that of most oils. Since persistent frothing occurred upon shaking some of the alcoholic extract of the fruit, a large percentage of the oil was unsaponifiable, and solanine-like compounds which belong to the sterol as well as alkaloidal class of organics are likely to be present; it is probable that in future work sterols will be isolated.

The results from the alkaloidal determination indicated the probable presence of alkaloids, such as Belladonna alkaloids, in the leaves and tops but indicated the absence of such alkaloids in the fruit. This does not mean that solanine-like compounds are absent from the leaves and tops or fruit. Since solanine-like alkaloids occur in plants as glycosides and are insoluble in chloroform and ether, they would not be included in this determination. Observations on the possible toxicity of the plant indicated the probability of the presence of poisonous substances. Since Chesnut (2) has experimental proof of the toxicity of the berries, *Solanum triflorum* should be considered as a poisonous plant until its toxicity is repudiated.

It is hoped that further chemical and pharmacological investigations will reveal the nature of the active constituent of various parts of the plant. Completion of the work which is in progress may indicate the possibility of some pharmacological action and justify the use of the plant in medicine.

REFERENCES

(1) Nuttall, T., "The Genera of North American Plants, and a Catalogue of the Species to the Year 1817," printed for the author by D. Heartt, Philadelphia, Vol. I, 1818, p. 128.

(2) Chesnut, V. K., "Principal Poisonous Plants of the United States," United States Dept. of Agr., Division of Botany, *Bull.* 20, 1898, p. 53; Chesnut, V. K., and Wilcox, E. V., "The Stock-Poisoning Plants of Montana," United States Dept. of Agr., *Bull.* 26, 1901, p. 125.

(3) Pammel, L. H., "A Manual of Poisonous Plants," The Torch Press, Cedar Rapids, Iowa, 1910–1911, p. 718.

(4) Fyles, F., "Principal Poisonous Plants of Canada," Dominion of Canada Dept. of Agr., *Bull.* 39, 1920, p. 88.

(5) Muenscher, W. C., "Poisonous Plants of the United States," The Macmillan Co., N. Y., 1939, p. 203

(6) Chesnut, V. K., and Wilcox, E. V., "The Stock-Poisoning Plants of Montana," United States Dept. of Agr., *Bull.* 26, 1901, p. 125.

(7) St. John, H., "Flora of Southeastern Washington and Adjacent Idaho," Student Book Corp., Pullman, Wash., 1937, p. xviii.

(8) "United States Pharmacopœa XI," Mack Printing Co., Easton, Pa., 1936, pp. 473, 474.

(9) Woodman, A. G., "Food Analysis," McGraw-Hill Book Co., New York, N. Y., 1931, p. 20.

(10) "United States Pharmacopœia XI," Mack Printing Co., Easton, Pa., 1936, p. 473.

(11) Dragendorff and Greenish, "Plant Analysis," G. E. Stechert and Co., New York, N. Y., 1921.

(12) "United States Pharmacopœia XI," Mack Printing Co., Easton, Pa., 1936, p. 474.

(13) *Ibid.*, p. 443.

(14) *Ibid.*, p. 193.

Solanum Triflorum Nutt. II. Histology*,†

By Leo A. Sciuchetti and R. L. McMurray

In a previous paper (1) the results of a preliminary investigation of *Solanum triflorum* Nutt. were reported. The available literature has revealed no histological investigation on this plant. In order to correlate results of the chemical investigation, a histological study of the plant was undertaken.

EXPERIMENTAL

Materials and Methods.—Month-old specimens of the plant grown in a laboratory window from seed gathered the previous fall were prepared for killing and fixing. The samples were divided into two portions and fixed in formalin-acetic acid-alcohol fixative and Lewitsky's (2) solution. The former fixative proved the more satisfactory. After removal of the fixative and complete dehydration the samples were embedded in paraffin. Consecutive transverse and longitudinal section 10 μ in thickness were cut with a microtome. A differential stain of aqueous safranin (1%) and fast green (1% in 95% alcohol) was used in staining. Slides were prepared from all parts of the plant but the stem and root, which were cut at 50 μ on a sliding microtome; stained with 2% aqueous safranin and counterstained with 1% fast green in clove oil. Camera lucida drawings were made from the various sections.

The Fruit of Solanum triflorum Nutt.—Structurally the fruit (Figs. 1, 2 and 3) is composed of three main portions: pericarp, placental tissue and seeds. The pericarp only has been studied. The outer epidermis of the fruit is covered by a relatively thick cuticle. The majority of the thin-walled epidermal cells are rectangular in shape in cross section, although polyhedral cells are frequently found. Stomata occur infrequently, the stomatal chambers being minute. Trichomes are lacking. Usually two to four layers of polyhedral thin-walled parenchyma cells separate the epidermis from the thick-walled collenchyma cells. The collenchyma are usually elongated with irregular thickening which is most prominent at the corner of the cells. Intercellular spaces occur infrequently within this area. Large thin-walled parenchyma cells also separate the collenchyma tissue from the characteristic stony bodies, which are composed of thick-walled stone cells. The inner parenchymatous cells contain numerous amyloplastids with enclosed starch grains, which are especially noticeable around the nucleus of each individual cell. A distinct hilum is noticeable in the majority of the starch grains. The vascular bundles within the pericarp are bicollateral. The inner and outer phloem strands are separated from the xylen elements by parenchyma-like cells. The inner polyhedral epidermal cells of the carpels are larger and thicker walled than those of the outer epidermis of these structures.

Characteristic Stone Bodies from the Fruit of Solanum triflorum Nutt.—A characteristic feature of the pericarp of the fruit is the presence of numerous stone bodies (Figs. 2 and 3). The stone cells from this region (Fig. 3, *A*, *st*) are thick walled, and plasmodesmata are well defined (Fig. 3, *B*). The presence of nuclei in many of these cells, even in the mature fruit, indicates that the cells are still alive.

Counts were made to determine the number of stone bodies and also the number of seeds in each fruit. Three lots from varied sources were used in the count. Results are given in Table I. The results obtained from these counts indicate that these stone bodies vary among the fruits of different plants as well as among the fruit of the same plant. There is no correlation between the number of stone bodies and the number of seeds in each fruit. The arrangement throughout the elliptical-shaped fruit is irregular, and the stone bodies are absent at the base of the fruit. It is possible that the stone bodies play an important role in the support and preservation of the fruit since they contribute to the hardness of the fruit. The dried stone bodies cannot be crushed by hand but require a hard, metallic instrument before they are crushed.

A comparison was made with the fruits from two other species of *Solanum*. An investigation of the dried fruits of *Solanum villosum* disclosed that similar bodies, which were approximately half the diameter of those from *Solanum triflorum*, were always situ-

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Presented to the Scientific Section, A. PH. A., Columbus meeting, 1943.

ated in the apex of the fruit. The number varied from one to three with a general average of two. Mr. Dwight Forsyth, Seed Analyst at the State College of Washington, states that he has failed to find stone bodies in the mature fruit of *Solanum nigrum* whereas an average of two was always found at the apex of the fruit of *Solanum villosum*.

Seeds of Solanum triflorum Nutt.—No histological work was attempted on the seeds. However, in making the counts it was noted that the seed coat and the seed itself contained a large amount of oil.

Perianth of Solanum triflorum Nutt.—Numerous glandular trichomes are found on the epidermis of the persistent perianth below the fruit. All trichomes are of the multicellular uniseriate non-branched type, and the glandular heads may vary from three or four to sixteen or more cells. Non-glandular hairs are found infrequently in this region.

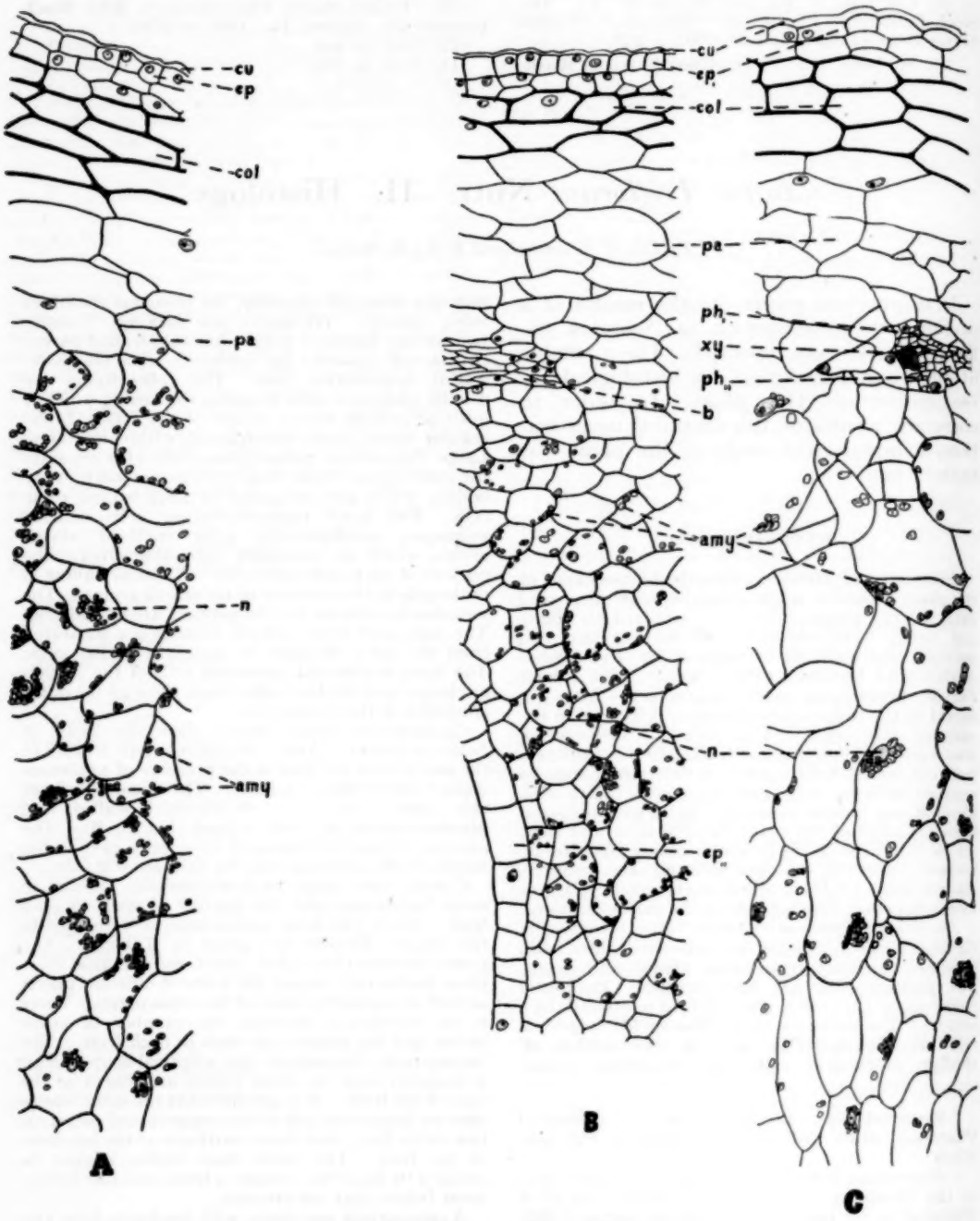

Fig. 1.—*Solanum triflorum* Nutt.

Sections through tissue (minus stone bodies) of three different fruits. *A*, C. S. of sector of mature fruit; *B*, L. S. of immature fruit; *C*, L. S. of mature fruit. *cu*, cuticle; *ep*$_{I}$, outer epidermis of the fruit; *col*, collenchyma; *pa*, parenchyma; *ph*$_{I}$, outer phloem; *ph*$_{II}$, inner phloem; *xy*, xylem; *b*, parenchyma cell of the bundle; *amy*, amyloplastids; *n*, nucleus of the cell; *ep*$_{II}$, inner epidermis of the fruit. ×190.

The epidermis is similar to that of the leaf as are the stomata although the stomatal chambers are considerably smaller in the perianth. An ill-defined palisade layer of cells occurs below the upper epidermis and the spongy parenchyma is of the aerechyma type. The arrangement of the vascular bundles corresponds to that of the leaf.

The Petiole of Solanum triflorum Nutt.—The petiole (Fig. 4) is slightly flattened, giving transverse sections an elliptical form. The epidermis of the petiole is less pubescent than that of the stem. Both glandular and nonglandular trichomes occur on the epidermis, but the former less frequently. The epidermal cells are irregular in shape and lack a cuticle. The chlorenchyma region is one cell in depth (although two or more cells may occur irregularly) and forms a continuous ring in section. Parenchyma of large irregular cells giving rise to numerous intercellular spaces and a few crystal sand cells compose the larger part of the cortical region. Abaxial and adaxial phloem strands are separated from each other by parenchyma cells of various sizes and shapes. The comparatively large pith is composed of large parenchyma cells. Crystal sand cells also occur in this area.

The Leaf of Solanum triflorum Nutt.—The lamina, or blade, of the leaf is thin, the chlorenchyma consisting of a single row of irregular palisade cells and a porous spongy region of three or four layers of cells (Fig. 5). The bicollateral bundle is similar, except as to size, to that of the stem. Both abaxial and adaxial phloem is present in the main laterals. Polyhedral parenchyma-like cells separate the abaxial and adaxial discontinuous phloem strands from the xylem elements. Both nonglandular and glandular trichomes occur on the surface of the leaf, the former being more prominent on the edges and ends of the

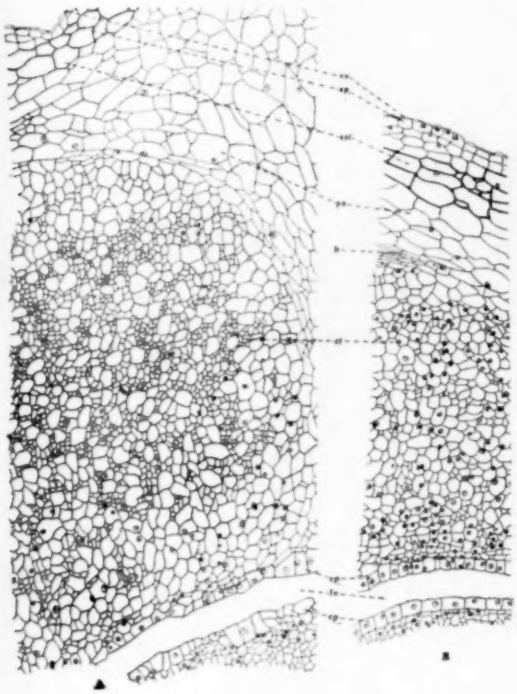

Fig. 2.—*Solanum triflorum* Nutt.

L. S. of sectors through two different fruits through stony body. *A*, nearly mature fruit; *B*, young fruit. *cu*, cuticle; ep_1, outer epidermis of fruit; *col*, collenchyma; *pa*, parenchyma; *b*, parenchyma cells near bundle; *st*, stone cells; ep_{II}, inner epidermis of fruit; *lo*, locule; ep_{III}, outer epidermis of the ovule. ×80.

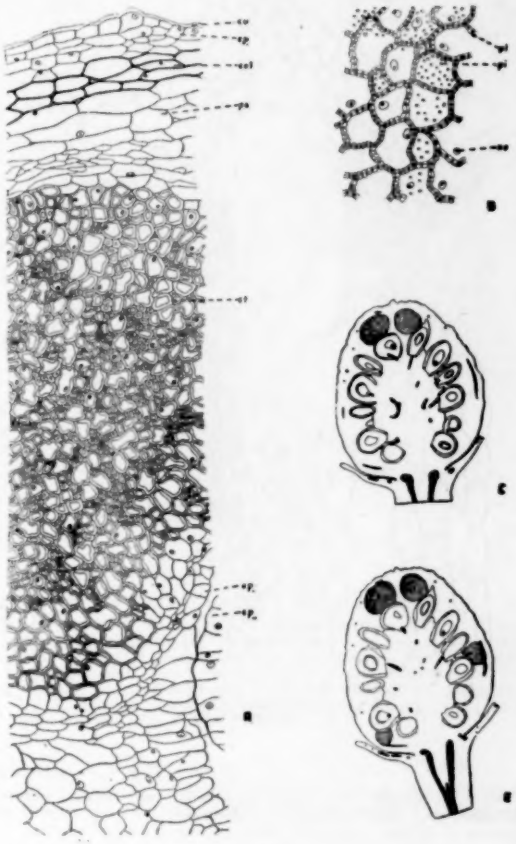

Fig. 3.—*Solanum triflorum* Nutt.

A, sector of C. S. of the mature fruit through stony tissue ×100. *cu*, cuticle; ep_1, outer epidermis of fruit; *col*, collenchyma; *pa*, parenchyma; *st*, stony tissue; ep_{II}, inner epidermis of fruit; ep_{III}, inner epidermis of ovary. *B*, C. S. through stony tissue ×225. *pl*, plasmodesmata and pits in side view; *pi*, pits in surface view; *nu*, nucleus. *C* and *E*, diagrammatic median L. S. of two fruits to show arrangement of stony bodies and vascular bundles ×4.0. The stony bodies are stippled; vascular bundles, solid.

TABLE I.—STONE BODIES AND SEED COUNT PER FRUIT

Fruit No.	Lot I		Lot II		Lot III	
	Stone Bodies	Seeds	Stone Bodies	Seeds	Stone Bodies	Seeds
1	12	36	11	72	15	60
2	13	87	8	71	11	68
3	13	70	14	65	15	58
4	10	92	11	61	13	52
5	15	21	11	42	14	60
6	13	50	9	75	14	67
7	13	92	11	60	11	58
8	8	50	12	59	11	50
9	10	82	12	96	12	60
10	12	53	14	66	13	54
Av.	11.9	63.3	11.3	66.7	12.9	58.7

leaf. Stomata are more numerous on the lower epidermis. The stomatal chambers are large. A cuticle is entirely lacking.

Stomata.—Stomata are more numerous in the lower epidermis of the blade and upper epidermis of the petiole of the leaf. Counts determined from fresh sections disclosed the following averages:

	Stomata per Sq. Mm.
Epidermis of:	
Lamina, upper	84.0
Lamina, lower	101.0
Petiole, upper	3.6
Petiole, lower	0.86
Stem	3.8

Numerous chloroplasts occur within the two guard cells of the stoma (Fig. 7). Usually three contiguous cells surround the guard cells, with four contiguous cells occurring less frequently.

Trichomes.—A study of the trichomes from all parts of the plant was made (Fig. 7). Both the glandular and nonglandular trichomes are of the multicellular, uniseriate, nonbranched type. Glandular hairs occur most abundantly on the epidermis of the perianth of the fruit. The glands of these trichomes may vary from three or four to many cells. The leaf epidermis bears both glandular and nonglandular hairs. This same condition occurs on the surface of the pedicel and peduncle, but the nonglandular type is more prevalent on these structures. The majority of the nonglandular trichomes are found on the surface of the stem.

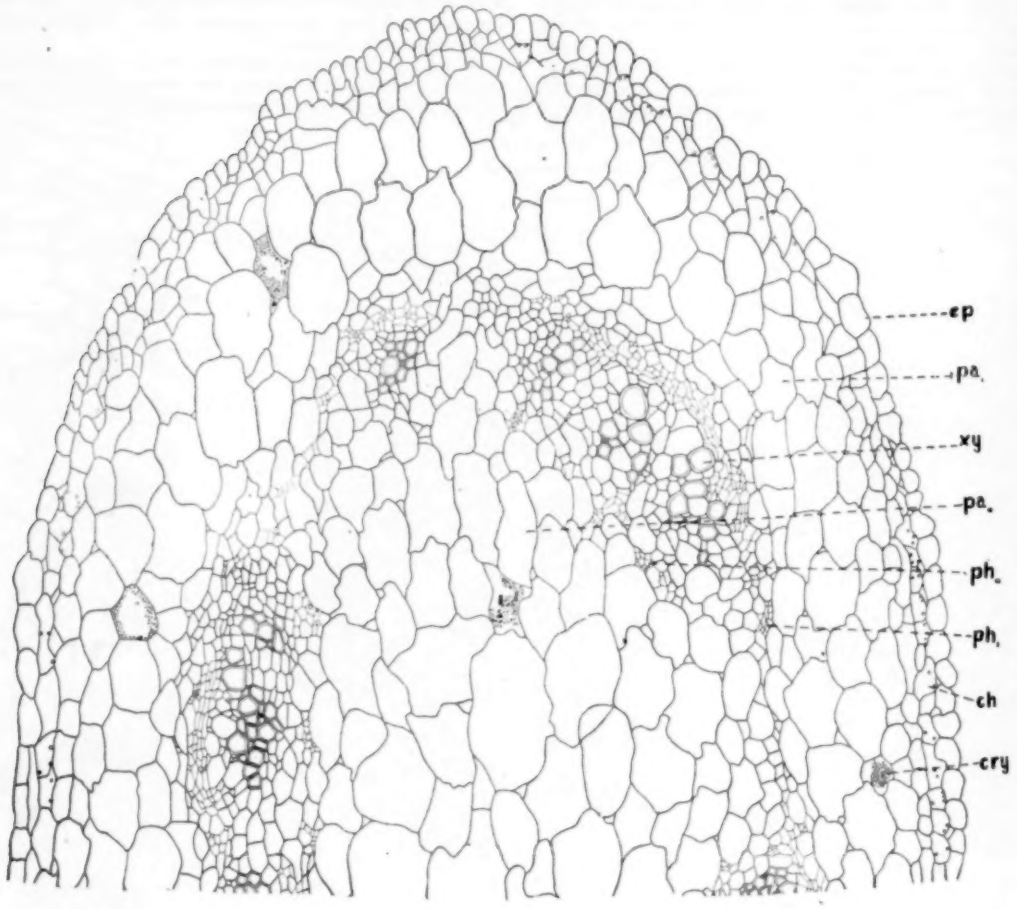

Fig. 4.—*Solanum triflorum* Nutt.

C. S. of petiole. *ep*, upper epidermis; pa_I, cortical parenchyma; pa_{II}, parenchyma of the pith; *xy*, xylem; ph_I, outer phloem; ph_{II}, inner phloem, *ch*, chlorenchyma, *cry*, crystal sand cell. ×90.

The Root of Solanum triflorum Nutt.—The root (Fig. 6) is a protostele. Xylem rays, usually one cell in width, were observed in month-old specimens. In the peripheral xylen of the older mature specimens the xylen rays are two cells in width and are composed of elongated thick-walled parenchyma. The ray cells are thick walled and are not readily distinguished in cross section throughout the plant. The scattered older phloem strands are separated from each other by parenchymatous cells, among which are crystal sand cells. A cambial zone and then undifferentiated xylem separates the phloem from the mature xylem. The endodermis is recognized by the presence of Casparian strips. Storage starch, especially surrounding the nuclei of each cell, is found in the endodermis and parenchyma of the pericycle.

The Stem of Solanum triflorum Nutt.—The stem has an amphiphloic siphonostele (Fig. 6). The inner phoem forms a ring of scattered phloem strands that are separated from the xylem and from each other by parenchymatous cells. Fibers frequently occur central to these phloem strands. A cambial region composed of flattened, thin-walled rectangular cells separates the outer phloem band from the xylem. Adventitious roots originate in the parenchyma of the phloem and pericycle. Large, which may be two or three cells in width. The parenchyma cells in the inner cortical region are large, and intercellular spaces are common. Some of the cells just external to the endodermis may contain starch and other inclusions.

The Peduncle of Solanum triflorum Nutt.—The structure of the peduncle (Fig. 8) closely resembles that of the pedicel (Fig. 9). Nonglandular trichomes are numerous on the epidermis, which is covered with a thick cuticle. Glandular hairs are

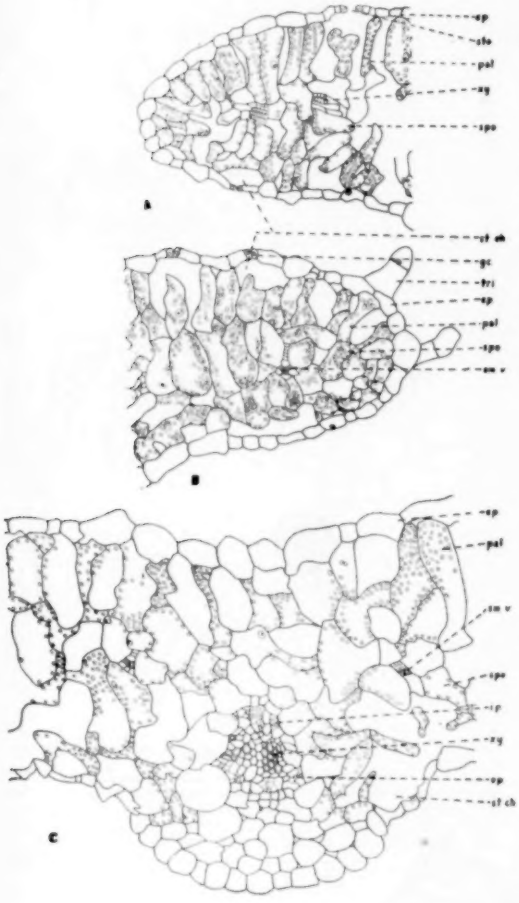

Fig. 5.—*Solanum triflorum* Nutt.

Sectors of C. S. of the leaf. *A*, *B*, opposite edges, *C*, through midrib. *ep*, upper epidermis; *sto*, stoma; *pal*, palisade; *spo*, spongy tissue; *st ch*, stomatal chamber; *gc*, guard cell; *tri*, trichome; *sm v*, small vein; *xy*, xylem; *ip*, adaxial phloem; *op*, abaxial phloem. ×100.

usually rounded, parenchyma cells with inclusions of storage starch, etc., and many crystal sand cells compose the pith. The endodermis is recognized by the numerous starch grains and inclusions. Fibers, singly or in groups, are arranged central to the endodermis throughout the pericycle.

A thick cuticle covers the epidermal cells. Stomata with minute stomatal chambers are found in the epidermis. Numerous large nonglandular trichomes occur. Glandular hairs are found less frequently. One layer of chlorenchyma cells forms a continuous band directly within the epidermis. Central to this is a continuous band of collenchyma

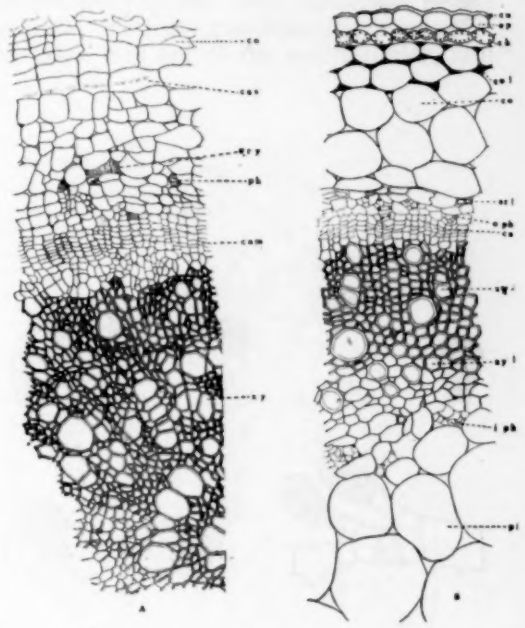

Fig. 6.—*Solanum triflorum* Nutt.

A, C. S. of the root; *B*, C. S. of the stem. *A*, *co*, cortex; *cas*, Casparian strip of the endodermis; *cry*, crystal sand cell; *ph*, phloem, *cam*, cambium; *xy*, xylem. *B*, *cu*, cuticle; *ep*, epidermis; *ch*, chlorenchyma; *col*, collenchyma; *co*, cortex; *scl*, sclerenchyma; *o ph*, outer phloem; *ca*, cambium; *xy 2*, secondary xylem; *xy 1*, primary xylem; *i ph*, inner phloem; *pi*, pith. ×90.

less frequent. The crotical region of the peduncle differs from that of the pedicel in that the intercellular spaces are smaller, storage starch is absent from the inner cortical region, and crystal sand cells are found in this region. Numerous sclerenchyma cells in the form of fibers occur within an ill-defined endodermal ring of cells.

The vascular arrangement of the peduncle is an amphiphloic siphonostele. The xylem band is considerable larger than that of the pedicel. Thin-walled small rectangular cambial cells form a continuous ring outside the xylem. The pith is composed of large irregularly shaped parenchyma cell with numerous crystal sand cells.

The Pedicel of Solanum triflorum Nutt.—The thin-walled epidermal cells are irregular in shape and are covered with a thick cuticle (Fig. 9). Numerous nonglandular trichomes arise from the epidermis. Glandular hairs are more sparsely distributed. Stomata, as expected, are likewise present. The cortical chlorenchyma region is composed of large, irregularly shaped cells forming a spongy parenchyma or aerenchyma type of tissue (Fig. 9). The intercellular spaces are very large. The inner

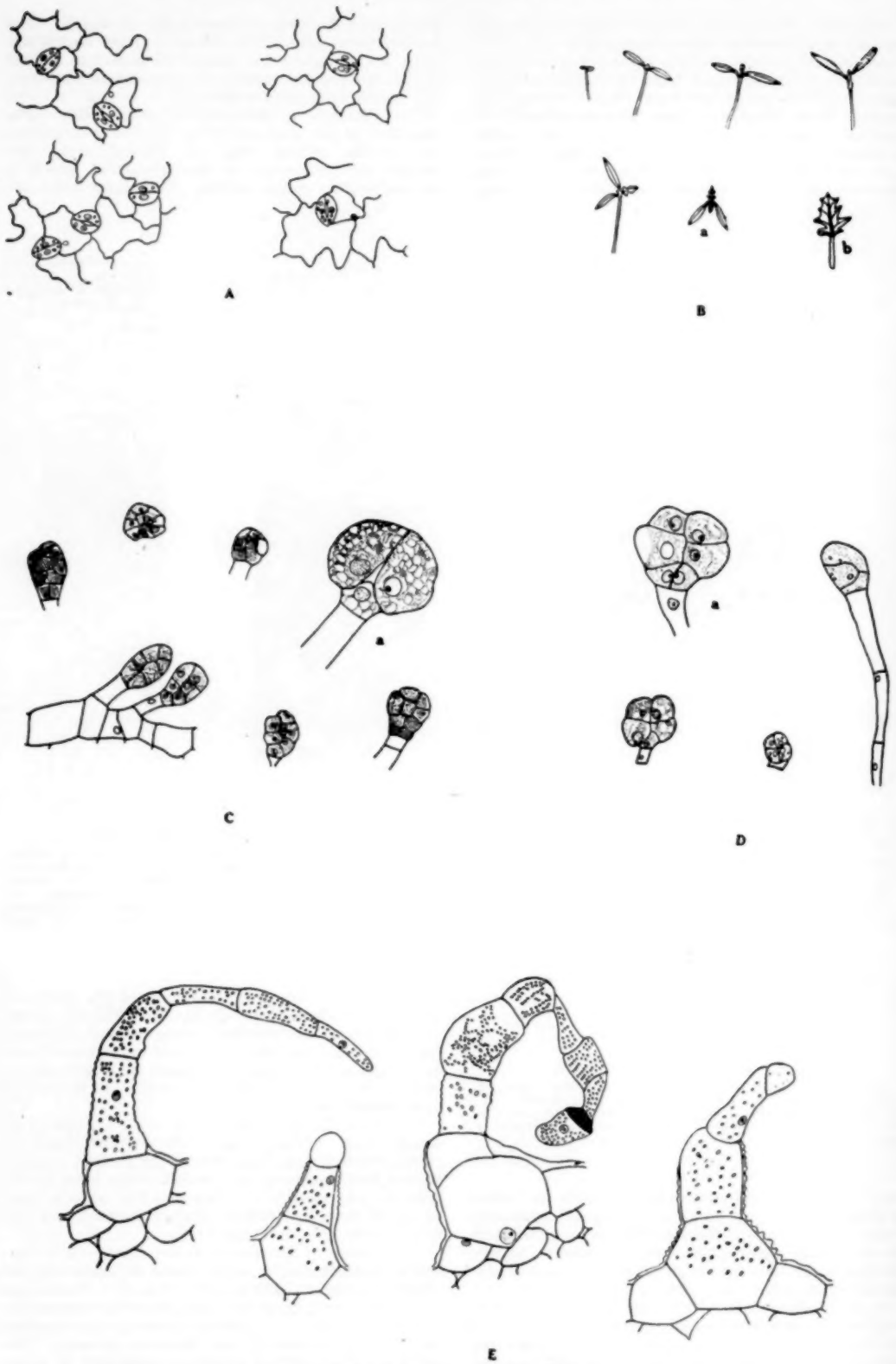

Fig. 7.—*Solanum triflorum* Nutt.

A, surface view of epidermis of the leaf showing stomata ×180. *B*, five stages in the development of leaves from the cotyledons; *a*, face or top view of the last stage, *b*, sketch of mature leaf showing venation ×1/4. *C*, glandular trichomes from the perianth of the fruit; *a*, ×390; all others ×180. *D*, glandular trichomes from the leaf; *a*, ×390, others ×180. *E*, nonglandular trichomes from the stem ×180.

cortical region is composed of large polyhedral parenchyma cells which are recognized by the presence of storage starch.

The pedicel has an amphiphloic dictyostele in which the bicollateral bundles are separated from each other by parenchyma. The endodermis is poorly defined. The pith is composed of large thin walled parenchyma cells. Crystal sand cells containing numerous micro-crystals occur throughout the medullary region.

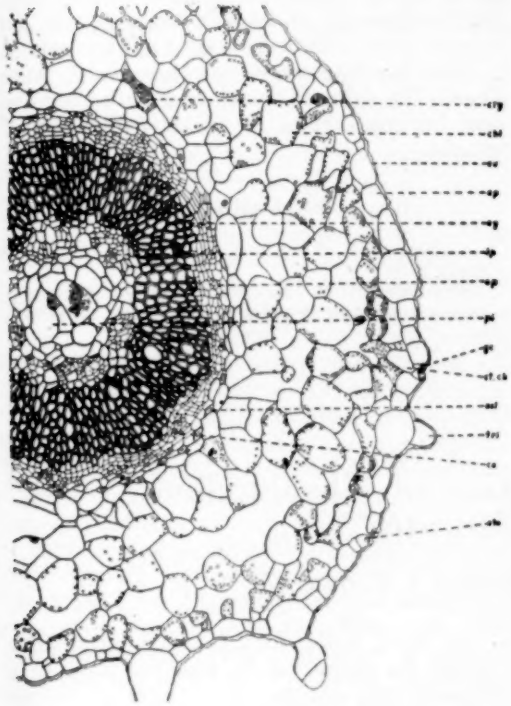

Fig. 8.—*Solanum triflorum* Nutt.

C. S. of peduncle form a mature fruit. *cry*, crystal sand cell; *chl*, chlorenchyma; *cu*, cuticle; *ep*, epidermis; *xy*, xylem; *ip*, inner phloem; *op*, outer phloem; *pi*, pith; *gc*, guard cell of stoma; *st ch*, stomatal chamber; *sto*, stoma, *scl*, sclerenchyma fiber; *tri*, nonglandular trichome; *ca*, cambium. ×90.

mon to all structures of the plant except the blades of the leaf and the fruit.

A thick cuticle is present on the epidermis of the above-ground portions of the plant except the petiole and blades of the leaf.

A study of the stomata and trichomes from all parts of the plant was made. Stomata counts were made on the upper and lower epidermis of the lamina, petiole and stem. The greatest number of stomata were found on the lower epidermis of the lamina. Both the glandular and nonglandular trichomes are of the multicellular, uni-

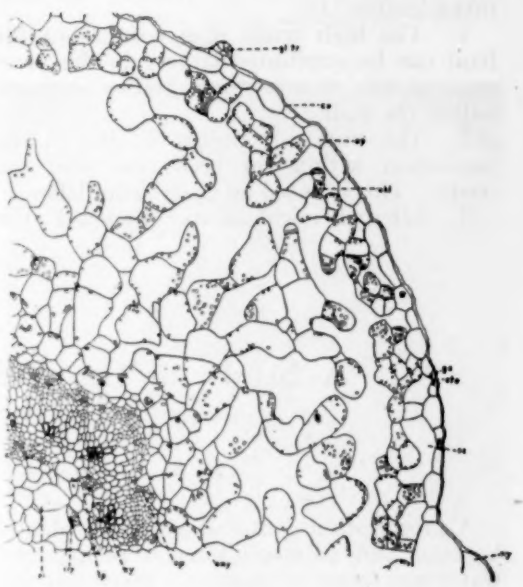

Fig. 9.—*Solanum triflorum* Nutt.

C. S. of pedicel near base of fruit. *gl tr*, glandular trichome; *cu*, cuticle; *ep*, epidermis; *chl*, chlorenchyma; *gc*, guard cell of stoma; *sto*, stoma; *sc*, stomatal chamber; *tri*, nonglandular trichome; *amy*, amyloplastid; *op*, outer phloem; *xy*, xylem; *ip*, inner phloem; *cry*, crystal sand cell; *pi*, pith. ×90.

SUMMARY AND CONCLUSIONS

A histological study of the entire plant of *Solanum triflorum* Nutt. has been presented.

A most helpful aid to the identity of the fruit of this plant is the presence of the numerous characteristic stony bodies. Counts showed that the number of these bodies in the same plant or different plants diverges. A comparison with two other species of *Solanum* was made. The microscopical identity of the fruits of *S. nigrum*, *S. villosum* and *S. triflorum* depends on the absence or presence of the stony bodies, the number of such bodies and their location.

Characteristic crystal sand cells similar to those found in *Atropa Belladonna* are common

seriate, nonbranched type. The multicellular, glandular hairs occur most abundantly on the perianth. The leaf epidermis, pedicel and peduncle bear both glandular and nonglandular hairs; the latter is more prevalent. The majority of the nonglandular trichomes are found on the surface of the stem.

The structure of the plant can be distinguished by the following features: the fruit by the presence of stony bodies; the perianth by the large number of glandular trichomes; the petiole by the presence of only a few nonglandular hairs, the presence of crystal sand cells, and the absence of cuticle and glandular trichomes on the epidermis; the blade of the leaf by the presence of a large number of glandular and non-

glandular trichomes and the absence of crystal sand cells; the root by the absence of both types of trichomes and the presence of stored starch, crystal sand cells, and Casparian strips in the endodermis; the stem by the presence of crystal sand cells, starch, trichomes and cuticle; the peduncle by the absence of starch grains; the pedicel by the presence of aeronchyma tissue. The variance in the amount and arrangement of the vascular bundles is also an aid to the identity of the various structures.

The following correlations may be made in comparing the results reported herein with the disclosures of the previous chemical investigation (1):

1. The high crude fiber content of the fruit can be attributed largely to the presence of the many stony bodies enclosed within the fruit.

2. The high oil content is due to the deposition within the seed coat and the seeds. This is based on observation alone.

3. Microchemical tests disclosed the presence of starch. Amyloplastids are found throughout the pericarp region of the fruit and other structures of the plant except the peduncle.

4. The high acid-soluble ash and the high alkalinity of the water-insoluble ash can be attributed largely to the presence of the characteristic crystal sand cells. The results of these two determinations were comparably lower for the fruit than other parts of the plant. This corresponds with the microscopical observations, since no crystal sand cells were found in the fruit.

The investigations of this plant have shown the value of correlating both chemical and botanical data in the identification of plants.

REFERENCES

(1) Sciuchetti, L. A., *et al.*, THIS JOURNAL, 9 (1944), 300.

(2) Lewitsky, G. A., *Bull. Applied Botany of Genetics and Plant Breeding* (U. S. S. R.), 23 (1931), 181.

A Study of the Reaction of Theophylline with Barbiturates*,†

By W. Mayo Higgins‡ and Melvin F. W. Dunker§

A prescription containing sodium phenobarbital and aminophylline in peppermint water was found to produce a precipitate on standing for a few hours. The melting point of the precipitate indicated that it was neither theophylline nor phenobarbital. The interest stimulated by this incompatibility lead to the present chemical investigation of the product and to a survey of the literature on the reaction products of barbiturates and nitrogenous compounds.

A complex of aminophylline and phenobarbital has been reported in which one mole of theophylline is reacted with one mole, or slightly more, of ethylenediamine and varying proportions up to 0.2 mole of phenobarbital, either in the presence or absence of solvent (1). However, this complex, unlike the substance precipitated above, had no definite melting point and was quite water soluble.

In 1910, Knoll and Co. (2) patented a process for preparing the reaction product of barbital and codeine or of barbital sodium and codeine hydrochloride. In the course of the next twenty-five years, a number of other companies and individuals extended the reaction to other alkaloids and other nitrogenous compounds with other 5,5-dialkyl- and 5-alkyl,5-aryl-barbituric acids, the reactants combining in a definite molecular ratio to form molecular compounds (3–31). These compounds were prepared by fusing the reactants or by dissolving the reactants in a suitable organic solvent from which the product crystallized. Patents have also been granted for molecular compounds prepared by the solution of hydrochlorides of the alkaloids and the sodium salts of the 5,5-disubstituted barbituric acids in water and allowing the product to crystallize (2–8).

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† The work here described is abstracted from one portion of the thesis presented by W. Mayo Higgins to the Graduate School of the University of Wisconsin, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, September, 1943.

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According to patents (5–11), the same molecular compound is obtained by crystallization from different organic solvents, by fusion, and by double decomposition of the salts of the reactants in aqueous solution, as long as the same ratio of reactants is retained. On the other hand, compounds may be formed containing 1 mole of the alkaloid and 1 mole of the barbiturate, 1 mole of the alkaloid and 2 moles of the barbiturate, or 2 moles of the alkaloid and 1 mole of the barbiturate, depending on the ratio of the reactants used (12, 13).

These molecular compounds are quite stable to air and to separation by solvent extraction. However, many of them are easily decomposed into their components by heating with dilute mineral acids (2, 7, 14). The patents claim for these compounds a combination and intensification of the physiological action of each component.

The reaction product of theophylline and phenobarbital is a white crystalline material (Fig. 1) melting at 250.7–251.7° C. (corr.). It is soluble in hot water, hot alcohol, dioxane and 5 per cent sodium hydroxide solution, slightly soluble in cold water, cold alcohol, ethyl acetate and 5 per cent hydrochloric acid, and insoluble in ether, acetone, carbon tetrachloride, chloroform, benzene and glycerine.

Analysis and carefully run reactions, recovering any unreacted materials, indicated the formation of a molecular compound containing 2 moles of theophylline and 1 of phenobarbital. This compound is readily decomposed into its components by sodium hydroxide, silver nitrate and boiling dilute hydrochloric acid. None of nineteen other barbiturates tried, including the closely related phenyl methyl barbituric acid, gave any reaction product under a variety of conditions, the reactants being recovered in 85 per cent yields or better in all cases. Under the same conditions, neither caffeine nor theobromine reacted with phenobarbital.

EXPERIMENTAL[1]

Isolation of the Material from the Prescription.—The following prescription was compounded, dissolving both solids separately and mixing the solutions:

Phenobarbital soluble	gr. xvi
Aminophylline	gr. 90
Aq. Peppermint, *q. s.*	fl. oz. viii

Within one hour, the solution contained a white crystalline precipitate which, when filtered off and dried, melted at 249–251° C., and after recrystallization from hot water melted at 250.7–251.7° C. A mixture of the unknown with theophylline showed a depression of the melting point.

Composition.—The same compound, melting at 250.7–251.7° C. after recrystallization from hot water or alcohol, and giving no depression of the melting point when mixed with the substance formed in the prescription, was obtained from the reaction of 1.0 Gm. of theophylline and 0.2 Gm. of sodium phenobarbital in 240 cc. of hot water or from 1.0 Gm. of theophylline and 0.2 Gm. of phenobarbital in 50 cc. of hot alcohol, indicating that neither the ethylenediamine nor the sodium ion is involved.

A sample of the material prepared in alcohol solution was dried overnight at 145° C. *in vacuo* and analyzed. The analytical results indicate an empirical formula of $C_{26}H_{30}N_{10}O_7$. This composition compares favorably with that of a molecular compound of 2 moles of theophylline and 1 mole of phenobarbital $(C_{26}H_{28}N_{10}O)_7$.

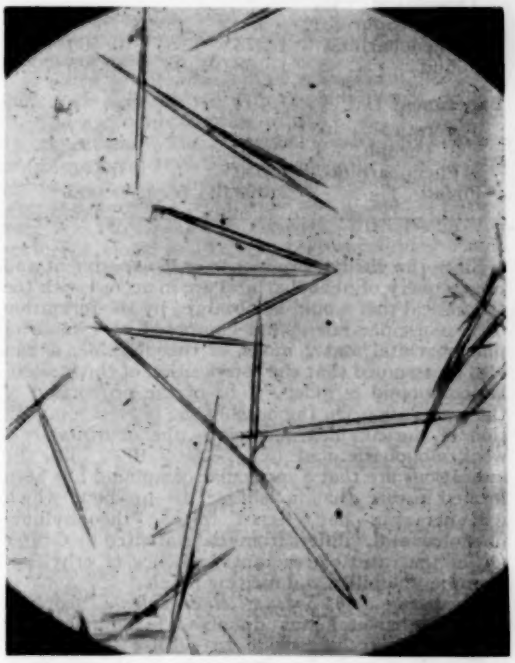

Fig. 1.—Thepohylline-phenobarbital (from Water). 100 ×.

Anal. Calcd. for $C_{26}H_{28}N_{10}O_7$: C, 52.8%; H, 4.73%; N, 23.6%. Found: C, 52.8%; H, 5.01%, N, 23.23%.

The composition of the product was further verified by the following two experiments carried out on a quantitative basis. In the first, the reactants were mixed in a molar ratio of 1:1 by dissolving 1.0 Gm. of phenobarbital in 50 cc. of hot alcoholic solution of 0.775 Gm. of theophylline. In the second, the reactants were mixed in a ratio of 1 mole of phenobarbital to 2 moles of theophylline by dissolving 1.0 Gm. of phenobarbital in 50 cc. of hot alcoholic solution of 1.55 Gm. of theophylline.

When the solutions had cooled, the crystals were filtered off and the mother liquids repeatedly concentrated and the precipitates removed until no more product crystallized from the solutions. The solutions were then evaporated to dryness and the residues extracted with boiling ether to dissolve any

[1] The melting points, unless otherwise specified, are corrected by comparison of the thermometer used against a set of Bureau of Standards Anschütz thermometers.

excess phenobarbital. There remained a small amount of ether-insoluble material in each case. The ether solutions were evaporated to dryness leaving a residue which was identified as phenobarbital. The weight and melting point were determined on each crystal crop and each residue. In each experiment, the fractions melting above 240° C. were combined and recrystallized from 95% alcohol to a constant melting point of 250.7–251.7° C. A mixed melting point of this material with the analyzed material gave no depression. The data are found in Table I.

TABLE I.—RATIO OF THE COMPONENTS IN THE MOLECULAR COMPOUND OF THEOPHYLLINE AND PHENOBARBITAL

	Wt. of Complex, Gm.	Recovered Phenobarb., Gm.
Experiment I:		
Theoretical for 2 Theoph. - 1 Phenobarbital	1.275	0.500
Found	1.168 (91.6%)	0.395 (79%)
Experiment II:		
Theoretical for 2 Theoph. - 1 Phenobarbital	2.550	0.000
Found	2.393 (93.5%)	0.030

Since the yield of product in each experiment and the recovery of phenobarbital are in accord with the theoretical that would be obtained by the formation of a molecular compound composed of 1 mole of phenobarbital and 2 moles of theophylline, it can also be assumed that the composition of the molecular compound is independent of the proportion of the reactants. On the basis of the proven composition of the complex, and since phenobarbital is a weak monobasic acid (K_{ion}. 3.89 x 10^{-8}) (32), the indications are that a molecular compound has been formed rather than a salt. This product, which will hereafter be referred to as "theophylline-phenobarbital," differs from that patented by Grüter (1) in the ratio of reactants, absence of ethylenediamine, solubility and melting point.

Treatment with Sodium Hydroxide.—Theophylline-phenobarbital was dissolved in sufficient 1.0 normal sodium hydroxide to furnish 3 moles of sodium hydroxide for each mole of theophylline-phenobarbital. To this solution was added 25 cc. of 95% alcohol. The white precipitate which formed was filtered off, and when dried to constant weight at 105° C. weighed 0.564 Gm. In the course of two hours, two additional crops of precipitate, totaling 0.086 Gm., were filtered from the hydroalcoholic solution. This precipitate (total weight, 0.650 Gm.) was identified as sodium theophyllinate.

The hydroalcoholic filtrate, after removal of the sodium theophyllinate, was acidified with concentrated hydrochloric acid and evaporated. Extraction of the residue (0.479 Gm.) with boiling ether yielded 0.262 Gm. of phenobarbital and 0.218 Gm. of theophylline-phenobarbital. The theoretical and actual yields of the products obtained from the treatment of 1.0 Gm. of theophylline-phenobarbital with 1.0 normal sodium hydroxide are found in Table II.

A comparison of the yields gives additional evidence of a combination in the ratio of 2 moles of theophylline to 1 mole of phenobarbital. The fact that the actual total is greater than the theoretical is believed to be explained by the fact that hydrated sodium theophyllinate holds water quite tenaciously (33).

Treatment with 10 Per Cent Hydrochloric Acid.—A 0.205-Gm. sample of theophylline-phenobarbital was heated on a steam bath with 15 cc. of 10% hydrochloric acid for one hour. By cooling and concentration, two crops of crystals, weighing 0.055 and 0.016 Gm. and melting at 174–176° and 173–175°, respectively, were obtained and identified as phenobarbital.

The solution was then evaporated on a steam bath and the residue (0.125 Gm.), after recrystallization

TABLE II.—PRODUCTS OBTAINED BY TREATING THEOPHYLLINE-PHENOBARBITAL WITH SODIUM HYDROXIDE

	Actual, Gm.	Theoret. for Theoph.-phenobarb., Gm.
Sodium theophyllinate	0.650	0.680
Phenobarbital	0.262	0.392
Theophylline-phenobarbital	0.218	...
Total	1.130	1.072

TABLE III.—PRODUCTS OBTAINED BY TREATING THEOPHYLLINE-PHENOBARBITAL WITH HYDROCHLORIC ACID

	Actual, Gm.	Theoret. for Theoph.-phenobarb., Gm.
Phenobarbital	0.071	0.080
Theophylline	0.125	0.125
Total	0.196	0.205

from alcohol, was identified as theophylline. The actual and theoretical yields of the products obtained from the treatment of 0.205 Gm. of theophylline-phenobarbital with 10% hydrochloric acid are found in Table III.

A comparison of these yields gives further evidence that the molecular compound contains 2 moles of theophylline and 1 mole of phenobarbital.

On standing in cold 10% hydrochloric acid, theophylline-phenobarbital gave no evidence of decomposition.

Treatment with Silver Nitrate.—An aqueous solution of silver nitrate was added to a hot aqueous solution of 1.0 Gm. of theophylline-phenobarbital. The white gelatinous precipitate which formed was filtered and washed free of silver nitrate. Considerable difficulty was experienced in washing the precipitate because of its gelatinous character. The precipitate was dried, powdered and extracted completely with boiling ether to remove any phenobarbital. A portion of the residue, after drying overnight at 110° C., was assayed for silver by ignition.

Anal.—Calcd. for silver theophyllinate, $AgC_7H_7O_2N_4$: Ag, 37.46%. Found: Ag, 37.45%; 37.39%.

The results compare favorably with the theoretical for silver theophyllinate.

A second portion of the silver theophyllinate was suspended in alcohol and the theophylline liberated with hydrochloric acid and identified by a mixed melting point.

The filtrate and washings from the silver theophyllinate were combined and 0.302 Gm. of pheno-

barbital recovered as described under the treatment of the complex with sodium hydroxide. The theoretical and actual yields of the products obtained from the treatment of theophylline-phenobarbital with silver nitrate are found in Table IV.

The low yield of phenobarbital is believed due to oxidation of some of the phenobarbital by nitric acid during the drying of the phenobarbital. The nitric acid was formed on acidification of the filtrate from the silver theophyllinate and was not washed out of the ether.

TABLE IV.—PRODUCTS OBTAINED BY TREATING THEOPHYLLINE-PHENOBARBITAL WITH SILVER NITRATE

	Actual, Gm.	Theoret. for Theoph.-phenobarb., Gm.
Silver theophyllinate	0.951	0.973
Phenobarbital	0.302	0.392
Total	1.253	1.365

Solvent Treatment.—Although phenobarbital is quite soluble in most of the organic solvents listed in the introduction and theophylline is not, theophylline-phenobarbital did not decompose into its components when treated with these solvents.

Experiments with Other Barbiturates and Theophylline.—Since it has been reported in the literature that the same nitrogenous compound, for example, quinine, will form molecular compounds with different 5,5-disubstituted barbituric acids (5, 7, 8, 15, 16, 20, 26), the reaction of theophylline with 5,5-disubstituted barbituric acids other than phenobarbital was attempted. The following 5,5-disubstituted acids were tested:

Diethyl	Isopropyl bromallyl[1]
Ethyl isopropyl	Diallyl
Ethyl *n*-butyl	Ethyl cyclohexenyl
Ethyl 1-methyl butyl	N-Methyl methyl cyclohexenyl
Ethyl isoamyl	Phenyl methyl[1]
Ethyl hexyl	N-Methyl phenyl ethyl[1]
Ethyl 1-methyl 1-butenyl	1,3-Dimethyl phenyl ethyl
Allyl 1-methyl butyl	Phenyl allyl[1]
Allyl isobutyl	Diphenyl[1]
Isopropyl allyl	Diphenyl hydantoin[2]

These experiments were carried out on a quantitative basis. A weighed amount of the 5,5-disubstituted barbituric acid was dissolved in 50 cc. of a hot alcoholic solution containing enough theophylline to give a molar ratio of 2 theophylline to 1 of disubstituted barbituric acid. On cooling the solution, crystals formed and were filtered off. The mother liquor was repeatedly concentrated and the precipitate removed until no more crystals separated from solution. The remaining solution was evaporated. When the barbiturate was easily soluble in ether, the residue was extracted with boiling ether to remove the barbiturate. When the barbiturate was insoluble or slightly soluble in ether, the residue was extracted with cold 10% hydrochloric acid to remove the theophylline. In either case, the extract was evaporated to dryness to recover the barbiturate or theophylline. The weight and melting point were determined on each crystal crop and each residue. The percentage recovery of the reactants and the melting points of the residues indicated that no reactions took place. Recrystallization of the fractions gave products melting at the melting point of theophylline or at the melting point of the respective barbiturate. Mixed melting points with the proper compounds gave no depression.

Since no products were obtained from theophylline and barbiturates other than phenobarbital by reaction in alcohol, and since some of the patents (5–12) reported products obtained by fusing the reactants, five of the 5,5-disubstituted barbiturates (diethyl, phenyl methyl, diphenyl, isopropyl bromallyl and N-methyl phenyl ethyl) were subjected to the following fusion procedure.

Sufficient theophylline was added to 0.5 Gm. of the barbiturate in a test tube to give a molar ratio of 1:1 and the mixture heated in a Wood metal bath until liquefied. The mixture was stirred, allowed to cool, and dissolved out with boiling alcohol. The recrystallization was then continued as described above. In no case were new products obtained by this treatment, and better than 85% of the reactants was recovered.

Reaction of Barbituric Acid with Theophylline.—Barbituric acid reacted with theophylline in alcohol to yield a product melting at 220.5–221.5° C. with decomposition. The analytical results indicated an empirical formula of $C_{11}H_{12}N_6O_5$. This composition compares favorably with that of a compound containing 1 mole of theophylline and 1 mole of barbituric acid ($C_{11}H_{12}N_6O_5$). However, since barbituric acid ($K_{ion.}$ 1.08×10^{-4}) is a stronger acid than acetic, this compound is probably a salt.

Anal.—Calcd. for $C_{11}H_{12}N_6O_5$: C, 42.85%; H, 3.89%; N, 29.17%. Found: C, 42.63%; H, 3.86%; N, 28.80%.

Experiments with Other Nitrogenous Compounds and Phenobarbital.—These experiments were carried out using caffeine, theobromine and diphenyl guanidine under the conditions described for theophylline and other barbiturates. With caffeine and theobromine, there was no reaction as indicated by better than 90% recovery of the reactants in each case. Neither could a product be obtained by fusing the reactants.

Diphenyl guanidine reacted with phenobarbital in alcohol to yield a product melting at 195.5–196° C. The analytical results indicated an empirical formula of $C_{25}H_{24}N_5O_3$. This composition compares favorably with that of a compound composed of 1 mole of diphenyl guanidine and 1 mole of phenobarbital ($C_{25}H_{22}N_5O_3$). However, since diphenyl guanidine is a strong base (34), this compound is probably a salt.

Anal.—Calcd. for $C_{25}H_{25}N_5O_3$: C, 67.74%; H, 5.64%; N, 16.15%. Found: C, 67.75%; H, 5.54%; N, 15.96%.

Suggested Application of the Reaction.—In view of the fact that nineteen barbiturates substituted in the 5-position with varying combinations of radicals failed to produce a reaction product, the following procedure is suggested as a means of identifying phenobarbital and of distinguishing theophylline from caffeine and theobromine. However, the possibility of other barbiturates forming molecular compounds with theophylline should be further exhaustively investigated.

Dissolve 0.1 Gm. of phenobarbital in 10 cc. of a hot alcoholic solution of 0.155 Gm. of theophylline. Cool the solution and filter off the crystals. After one crystallization from alcohol, the crystals melt at about 250° C.

[1] Kindly supplied by H. A. Schonle of Eli Lilly and Co.

[2] Although not a barbiturate, diphenyl hydantoin was included because of its similarity in structure.

SUMMARY

1. Sodium phenobarbital reacts with aminophylline in aqueous solution to form a product melting at 250.7–251.7° C. The same product can be formed by allowing phenobarbital and theophylline to react in alcoholic solution. The product contains 2 moles of theophylline and 1 mole of phenobarbital and the ratio of components is independent of the proportion of the reactants.

2. Since there are 2 moles of theophylline for each mole of phenobarbital, and since phenobarbital is a weak monobasic acid, it is believed that the product is a molecular compound rather than a salt.

3. The compound is decomposed into its components by sodium hydroxide, silver nitrate and boiling hydrochloric acid.

4. None of nineteen other 5,5-disubstituted barbituric acids reacted with theophylline under the same conditions or by fusion with theophylline.

5. Barbituric acid reacts with theophylline in a molar ratio of 1:1 to produce a compound melting at 220.5–221.5° C. However, because barbituric acid is a comparatively strong acid, this compound is believed to be a salt.

6. Phenobarbital failed to react with caffeine or theobromine, but did react with diphenyl guanidine in a molar ratio of 1:1 to produce a compound melting at 195.5–196° C. However, because diphenyl guanidine is a strong base, this compound is believed to be a salt.

7. Because of the apparent specificity of the reaction of theophylline with phenobarbital forming a molecular compound, it is suggested that the preparation of "theophylline-phenobarbital" could be used for the identification of phenobarbital and as a means of distinguishing theophylline from caffeine and theobromine.

REFERENCES

(1) U. S. Patent 2,017,279 (Oct. 15, 1935).
(2) German Patent 239,313, *Frdl.*, 10 (1913), 1223.
(3) British Patent 306,905 (May 26, 1930).
(4) British Patent 416,273 (Sept. 13, 1934).
(5) German Patent 249,908, *Frdl.*, 11 (1915), 988.
(6) German Patent 291,421, *Ibid.*, 12 (1917), 754.
(7) U. S. Patent 1,185,637 (June 16, 1916).
(8) Swiss Patent 62,732, *C. A.*, 8 (1914), 2225.
(9) U. S. Patent 1,494,127 (May 13, 1924).
(10) U. S. Patent 1,798,556 (Mar. 21, 1931).
(11) U. S. Patent 2,014,866 (Sept. 17, 1935).
(12) British Patent 447,245 (May 14, 1936).
(13) Swiss Patent 77,194, *C. A.*, 12 (1918), 2111.
(14) U. S. Patent 1,459,347 (June 19, 1923).
(15) Swiss Patent 62,733, *C. A.*, 8 (1914), 2225.
(16) Swiss Patent 76,996, *Ibid.*, 12 (1918), 2111.
(17) Swiss Patent 76,997, *Ibid.*, 12 (1918), 2111.
(18) Swiss Patent 77,193, *Ibid.*, 12 (1918), 2111.
(19) Swiss Patent 77,195, *Ibid.*, 12 (1918), 2111.
(20) Swiss Patent 77,196, *Ibid.*, 12 (1918), 2111.
(21) Swiss Patent 77,197, *Ibid.*, 12 (1918), 2111.
(22) Swiss Patent 77,198, *Ibid.*, 12 (1918), 2111.
(23) Swiss Patent 77,199, *Ibid.*, 12 (1918), 2111.
(24) Swiss Patent 77,200, *Ibid.*, 12 (1918), 2111.
(25) Swiss Patent 77,201, *Ibid.*, 12 (1918), 2111.
(26) German Patent 247,188, *Frdl.*, 11 (1915), 987.
(27) German Patent 586,245, *C. A.*, 28 (1934), 578.
(28) German Patent 631,097, *Ibid.*, 30 (1936), 6136.
(29) U. S. Patent 1,478,463 (Dec. 25, 1923).
(30) Lusignani, G., *Ann. chim. farm.* (1939), 64; through *C. A.*, 34 (1940), 2382.
(31) Pfeiffer, P., and Seydel, R., *Z. physiol. Chem.*, 176 (1928), 1.
(32) Krahl, M. E., *J. Phys. Chem.*, 44 (1940), 449.
(33) Kruger, M., and Schmid, J., *Z. physiol. Chem.*, 36 (1902), 1.
(34) Merck Index, ed. 5, 1940, p. 204.

The Effect of Storage on the Activity of Defatted Ergot with Various Moisture Contents*

By J. Allen Reese†

Defatted ergot has been proposed for inclusion in the U. S. P. XII as the most satisfactory dosage form for ergot when the action of all the alkaloids of the drug is desired. A defatted ergot is official in the British Pharmacopœia (1932). Evidence has been accumulating to the effect that such a preparation is a relatively stable product. Christensen and Reese (1) concluded that moisture is the principal factor in the deterioration of whole ergot. The author considered it desirable to ascertain the effects of different moisture contents on the activity of defatted ergot.

Zschiesing and Bombelon (2) and Perret (3) suggested defatting as a means of preservation of ergot. Pedersen (4), (5) reported that defatted ergot was more stable than ergot. Corran and Rymill (6) kept samples of defatted ergot with moisture content of 10 per cent for seven and seventeen months. The former deteriorated 3 per cent, the latter 12 per cent.

EXPERIMENTAL

Material.—Four lots of ergot (30 Kg.) were used in this investigation. Lots 1 and 2 (16 Kg.), obtained in 1936, were labeled Spanish Ergot. Lots 3 and 4 (14 Kg.), obtained in 1937, were labeled Portuguese Ergot. The author was informed by the importer that these lots were from large shipments consisting of several tons, and that the majority of the Portuguese Ergot was of Spanish origin.

Preparation and Storage.—Representative samples from each lot were reduced to a No. 60 powder and percolated with Purified Petroleum Benzin to remove the fat. The defatted ergot was dried at a temperature not exceeding 40° C. The moisture content was determined by drying in an oven at 100° C., and the alkaloidal content determined by the method given below. The defatted ergot was placed in glass bottles and stoppered with corks dipped in paraffin, and stored at room temperature.

Assay Procedure.—Transfer 12 Gm. of defatted ergot to a stoppered flask, add 120 cc. anesthetic ether and set aside for ten minutes. Add 0.6 Gm. light magnesium oxide diffused in 20 cc. of water and shake in a mechanical shaker for one hour. Add 1.2 Gm of powdered tragacanth, shake vigorously, set aside ten minutes and filter through cotton 100 cc. of the ethereal solution, representing 10 Gm. of the defatted ergot. Transfer to a separatory funnel and shake with four successive 10-cc. portions of a 1% *w/v* solution of tartaric acid in water. A fifth extraction with a 5-cc. portion of the acid should give no precipitate with Mayer's reagent. If a precipitate is formed, continue the extraction with 5-cc. portions until a negative test is obtained. Mix the aqueous liquids, transfer to a porcelain dish, remove the dissolved ether by gentle warming on a water bath in a current of air and add sufficient water to produce 50 cc. or other suitable volume. Mix 1 cc. with 2 cc. of the Allport-Cocking reagent (7) and let stand ten minutes. In the same manner, mix 1 cc. of 0.012% solution of Ergotoxine Ethanesulfonate in 1% *w/v* tartaric acid in water (1 cc. of this solution contains the equivalent of 0.0001 Gm. of anhydrous ergotoxine) with 2 cc. of the Allport-Cocking reagent, and let stand for the same length of time. Determine the ratio of the color intensities by comparing them in a suitable colorimeter. The color produced by 1 cc. of 0.012% solution of Ergotoxine Ethanesulfonate is equivalent to that produced by 0.0001 Gm. of total alkaloids under identical conditions. The acid solution of the alkaloids should be suitably diluted so that the color, produced during the test, does not differ by more than 20% from that produced in the solution of Ergotoxine Ethanesulfonate.

Time may be saved by preparing dilutions of the acid solution of the alkaloids by mixing 1 part with 1, 2, 3, 4 and 5 parts of water, and mixing 1 cc. of each of these with 2 cc. of the Allport-Cocking reagent.

Allport and Cocking (7) have insisted on the use of pure anesthetic ether, to avoid oxidation of the alkaloids.

RESULTS

ASSAY OF DEFATTED ERGOT BEFORE AND AFTER STORAGE FOR FIVE YEARS

Lot No.	Moisture Content, %	Alkaloids, %	Alkaloids After Storage, %	Loss of Alkaloids, %
1	7.9	0.340	0.288	15.3
2	10.1	0.311	0.208	33.1
3	12.6	0.285	0.226	20.7
4	13.2	0.313	0.247	21.1

SUMMARY

Four lots of defatted ergot with moisture contents of 7.9, 10.1, 12.6 and 13.2 per cent were assayed for alkaloidal content and stored for five years. The effect of the storage on the alkaloidal content was then determined. The per cent of deterioration of alkaloids was 15.3, 33.1, 20.7 and 21.1 per cent, respectively. From previous work on whole ergot a correlation between deterioration and moisture content was expected. A correlation is shown between three of the lots; however, lot 2 deteriorated more than any of the others. No explanation is offered for this. The effect of storage on defatted ergot with lower moisture contents should be studied.

* Received Nov. 15, 1943, from the School of Pharmacy, University of Kansas, Lawrence, Kan.

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REFERENCES

(1) Christensen, B. V., and Reese, J. A., THIS JOURNAL, 28 (1939), 343.

(2) Zschiesing, G., *Pharm. Ztg.*, No. 49, 51; through *Am. J. Pharm.*, 53 (1881), 457.

(3) Perret, E., *Bull. gén. de thérap.*, 102 (1882), 202; Barger, G., "Ergot and Ergotism" Gurney and Jackson, London, 1931, p. 212.

(4) Pedersen, O. C., *Norg. Apotekerforen. Tids.*, 39 (1931), 49; through *C. A.*, 26 (1932), 3873.

(5) Pedersen, O. C., *Norg. Apotekerforen. Tids.*, 40 (1932), 49; *C. A.*, 26 (1932), 3873.

(6) Corran, P. F., and Rymill, F. E., *Quart. J. Pharm. Pharmacol.*, 8 (1935), 337.

(7) Allport, N. L., and Cocking, T. T., *Ibid.*, 5 (1932), 341.

Color Reactions and Stability of Iron Ascorbate*

By Louis Freedman and Ann Sack

The possibilities of using iron ascorbate as a therapeutic form of iron have led to several attempts to prepare this compound in pure form. A review of the rather meager literature on this compound indicates considerable divergence of opinion regarding not only its method of preparation but also its chemical and physical stability.

First mention of iron ascorbate in the literature was made in 1934 by Szent-Györgyi (1) who described a color reaction between ascorbic acid and ferrous sulfate. Szent-Györgyi added ferrous sulfate to an alkaline solution containing 1.0% of ascorbic acid, which mixture, on exposure to air, became dark violet in color. He explained the color reaction as being due to an oxide of the ferro-vitamin complex, since it could be reduced to the leuco compound by a small amount of hydrosulfite. Two years later, Maurer and Schiedt (2) also mentioned that, if ascorbic acid and powdered iron were allowed to react in the absence of air, a colorless solution of iron ascorbate was obtained. To exclude air, they carried out the reaction under toluene.

While several patents have been issued to inventors describing different methods of preparing iron ascorbate, we have been unable to find any data which might indicate that colorless iron ascorbate can actually be obtained in pure form. U. S. patent No. 2,260,870 issued to S. L. Ruskin and French patent No. 805,335, issued to Arloing, Morel and Josserand deal with colored iron ascorbate. Ruskin prepared iron ascorbate by adding ferric chloride to a sodium hydroxide solution of ascorbic acid and precipitated the product by addition of more sodium hydroxide. He obtained a dark brown or black substance. The French authors prepared the product by adding ferrous or ferric chloride to an alkaline solution of ascorbic acid and precipitated the iron ascorbate with anhydrous alcohol. They considered the product to be either a complex salt of ferrous and ferric ascorbate or the oxidation product of this complex salt.

British patents Nos. 472,531 and 486,757 relate to the manufacture of "stable" (colorless) ferrous compounds of ascorbic acid. The latter patent claims the preparation of a permanent ferro compound by treating ferrous salts, under slightly acid or neutral conditions, with ascorbic acid or alkali or alkaline-earth salts thereof. The iron ascorbate compound so produced in a slightly acid or neutral medium is said to be colorless or only slightly colored. The iron is claimed to be in a ferrous state and not easily oxidizable.

When iron ascorbate is freshly prepared according to the method of Maurer and Schiedt, it apparently has strong antiscorbutic properties as described by Gould and Schwachman (3). But the activity decreases with the decomposition of the product according to Friend (4) who found that the product may have greater therapeutic value for iron medication than for its antiscorbutic activity.

EXPERIMENTAL

The divergence of opinion regarding methods of preparing iron ascorbate and its chemical properties have led us to make a more extended investigation of this product. We have prepared iron ascorbate by the various methods suggested in the above-described patents, but in no case were we able to isolate a pure iron ascorbate either in colorless or colored form. We have, on the other hand, observed that the color of the product depends on the degree of acidity present in the final solution of the iron ascorbate and that a violet-colored product was always

* Received March 6, 1944, from the Research Laboratories of U. S. Vitamin Corporation, New York, N. Y.

obtained when the reaction of the mixture was less acid than *p*H 2.9.

Iron ascorbate prepared by reacting 1.8 Gm. of ascorbic acid dissolved in 10 ml. of water with 1 Gm. of pure metallic iron in the form of iron filings showed the following shades of color in the solution at different degrees of acidity (see Table I).

TABLE I

Color	*p*H
Light greenish yellow (almost colorless)	1.5
Light reddish yellow	2.9
Light reddish violet	3.4
Red-violet–brownish violet	3.8
Dark violet	5.6

Since the iron of the iron ascorbate, formed by the reaction of 1 part of iron filings and 1.8 parts of ascorbic acid previously dissolved in 20 parts of water, was obviously in the ferrous state (hydrogen gas was evolved during the reaction) it appeared rather doubtful that the violet color formed could have been the result of an oxidation complex. As the acid was used up by the iron, the solution became violet even during strong evolution of hydrogen gas. On the other hand, as long as the solution was strongly acid, it remained colorless even on decanting the solution from the iron and addition of hydrogen peroxide. The above observations appeared to indicate that the violet color formation was most likely a function of ionization influenced by acidity rather than an oxidation of the ferrous ascorbate or its complex.

To determine further whether the effect of acid in extinguishing the color was due to the decomposition of the iron ascorbate to free ascorbic acid and the iron salt of the added acid, we added different acids in excess, including ascorbic acid, to a violet-colored solution of iron ascorbate prepared as described below. The results of these acid titrations given in Table II, indicate that the dispatch of the pigment is not due to double decomposition but is definitely a function of acidity. The fact that excess of ascorbic acid itself, sufficient to bring the acidity to *p*H 2.9 or lower, will decolorize the solution confirms this point of view.

TABLE II

Acid Used	No. of Ml. Necessary to Decolorize 5 Cc. of a Ferrous Ascorbate Solution[a]	*p*H of Decolorized Solution
HCl *N*/1	1.0 ml.	2.6
H_3PO_4 *N*/1	2.25 ml. colorless but turbid	2.6
H_3PO_4 *N*/1	5.9 ml. colorless and clear	1.7
Ascorbic acid 20% *w*/*v*	10.7 ml.	2.9

[a] This colored iron ascorbate was prepared by dissolving 1.8 Gm. ascorbic acid and 1.6 Gm. barium hydrate in 25 ml. water and adding 1.4 Gm. of ferrous sulfate in 15 ml. water and filtering off the barium sulfate precipitate which formed. The *p*H of this solution was 5.2.

That the formation of the colored pigment may also be related to ionization is shown by the following experiment. A colorless solution of ferrous ascorbate was prepared by reacting a very dilute solution of barium ascorbate with a dilute solution of ferrous sulfate in an anaerobic atmosphere. On exposure to air for a few minutes, the solution turned violet color, but on further dilution with water, the color disappeared. However, when the ferrous ascorbate was prepared by reacting a concentrated solution of barium ascorbate with ferrous sulfate with exclusion of air, a dark violet solution resulted. The color could readily be dispatched by acidification or further dilution. By adding a colorless solution of ferrous ascorbate to alcohol, the mixture turned violet but when the alcohol was made strongly acid with HCl, no color formed.

Some observations regarding the stability of metallic and organic compounds of ascorbic acid have been previously reported. Maurer and Scheidt (2) reported that the violet-colored iron ascorbate solution became brown on standing and deposited ferrous oxalate crystals after ten days standing. Jurist and Christiansen (5) examined various solutions of ascorbates of inorganic and organic bases, and in each case found evidence of oxalate formation. Ghosh and Rakshit (6) attributed the formation of oxalic acid to autoxidation of ascorbic acid. Ghosh (7) reported, however, that this autoxidation could be prevented by addition of sulfhydril compounds such as glutathione, or cystine or thiolactic acid to the ascorbic acid solution.

We observed the formation of ferrous oxalate in solutions of colored ferrous ascorbate as well as acidified colorless solutions after four days' standing. We were, however, unable to confirm Ghosh's observations with respect to the inhibiting effect of cystine on the formation of oxalates.

The formation of ferrous oxalate may be masked because of its solubility in acid solutions and because of the fact that it also decolorizes dichlorophenol-indophenol dye, extensively used for titrating ascorbic acid, and thus may be mistaken for the latter. This confirms the observation of Basu and Nath (8) who reported the disturbing effect of iron oxalate in the titrimetric estimation of ascorbic acid with indophenol dye.

By actual titration of weighed amounts of purified ferrous oxalate we have found that the latter salt is only about one-eighteenth as active, weight for weight, as ascorbic acid in decolorizing the indophenol dye.

SUMMARY AND CONCLUSIONS

Iron (ferrous) ascorbate, prepared by several different methods, is not a stable compound. When at first formed, particularly in strongly acid or very dilute aqueous solution, it appears to be a colorless substance, but on standing or on isolation from a neutral or weakly acid medium, it gradually becomes pigmented, finally turning a dark violet color. While this colored form of iron ascorbate, particularly in an alkaline medium, is undoubtedly a complex, the nature of which is as yet unknown, it readily reverts to the colorless form on acidification to a *p*H of 2.9 or less. Experimental evidence has been presented to show that the formation of the complex is not necessarily a function of oxidation but may rather be a function of *p*H.

Iron ascorbate in aqueous solution in the colorless or pigmented form is chemically unstable in that it gradually decomposes to form iron oxalate which is a strong reducing agent. The presence of iron oxalate in iron ascorbate or other metallic ascorbates may

be masked when titrated with dichlorophenol-indophenol dye, since the former will also reduce the dye but to an extent about one-eighteenth of that of ascorbic acid.

REFERENCES

(1) Szent-Györgyi, A., *Z. physiol. Chem.*, 225 (1934), 168.
(2) Maurer, K., and Schiedt, B., *Biochem. Z.*, 285 (1936), 67.
(3) Gould, B. S., and Schwachman, H., *J. Biol. Chem.*, 151 (1943), 452.
(4) Friend, D. G., *New Engl. J. Med.*, 219 (1938), 910.
(5) Jurist, A. E., and Christiansen, W. G., *Am. J. Pharm.*, 111 (1939), 347.
(6) Ghosh, J. C., and Rakshit, P. C., *Biochem. Z.*, 299 (1938), 394.
(7) Ghosh, J. C., *J. Indian Chem. Soc.*, 15 (1938), 1.
(8) Basu, K. P., and Nath, M. C., *Ibid.*, 15 (1938), 133.

The Chromatographic Purification and Some Properties of Atebrin Base*

By Llewellyn H. Welsh†

In the course of toxicity studies on atebrin[1] dihydrochloride [6-chloro-9-(4-diethylamino-1-methylbutyl)amino-2-methoxyacridine dihydrochloride dihydrate], it was necessary to prepare a highly purified atebrin base. The chromatographic method of purification was used advantageously for this purpose by allowing the free base to be adsorbed on activated alumina from a benzene solution. The substance thus purified was crystallized from ethanol-water.

Atebrin base was first reported in the literature by Magidson and Grigorovskiĭ (1), who described it as prismatic needles melting at 86–88°. Chelintsev and Osetrova (2) more recently have referred to a melting point of 86°. The only analyses reported by Magidson and Grigorovskiĭ were chlorine determinations which afforded a value of 8.6 per cent. Since the percentage found was compared with a theoretical value based on a $C_{23}H_{30}ClN_3O$ formula, it is obvious they considered the substance to be an anhydrous base. Their analytical results, however, agree more closely with 8.48 per cent calculated for a monohydrate than with 8.87 per cent for the anhydrous base. That the crystalline substance is a monohydrate has been recognized in the laboratories of the Winthrop Chemical Company[2] and, independently, in this laboratory. Its melting range was found to depend somewhat on the rate of heating in the determination during which progressive loss of water probably occurs. In this laboratory it has not been possible to prepare the anhydrous base in a crystalline condition. Removal of water from the hydrate, at and above room temperature, always has yielded a highly viscous material which when exposed to air at room temperature gained weight rapidly and which very slowly crystallized as the hydrate.

Atebrin base, like the dihydrochloride, slowly acquires a brownish or orange discoloration of areas exposed to sunlight. That several substances are formed by ultraviolet irradiation was demonstrated chromatographically.

* Received Jan. 29, 1944, from the U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.
† Appreciation is expressed to Drs. T. G. Klumpp and B. Puetzer of the Winthrop Chemical Company for a supply of atebrin dihydrochloride and seed crystals of atebrin base.
[1] There are several variations of spelling. The drug is also known as quinacrine (U. S. P. XII) chinacrin, acriquine, mepacrine, italchin.
[2] Private communication from Dr. A. E. Sherndal.

EXPERIMENTAL

Liberation and Crystallization of the Free Base.—Thirty grams of commercial atebrin dihydrochloride dihydrate was dissolved in 350 cc. of hot water. After the solution had cooled somewhat, 100 cc. of benzene was added, the mixture made alkaline with a slight excess of 20% sodium hydroxide and immediately shaken vigorously. After several extractions

with warm benzene (until the solvent was no longer yellow) the bulked extract was washed with water, dried superficially with sodium sulfate, filtered, and most of the benzene distilled off. The remaining solvent was removed by repeatedly heating on the steam bath and placing in a vacuum. The residue was dissolved in 100 cc. of hot 95% ethanol, 45 cc. of water added, the solution cooled to 40–45°, seeded and allowed to cool, with occasional stirring, to a temperature of 10° during an interval of several hours. This crystallizing procedure was found necessary to prevent deposition of the product initially as oily droplets. Occasionally it was necessary to add small amounts of additional ethanol during the cooling to 40° to avoid an oily turbidity or conversion of the seed to an oil. This may have been due to the presence of more than traces of benzene in the residue. The product was sucked fairly dry on a Büchner and left three to four hours in a desiccator at a pressure of *ca.* 40 mm. over calcium chloride. The quantity of material so obtained amounted to 90–95% of the theoretical. Specimens in capillary tubes placed in a bath at 75° melted at 82.5–85.5° (corr.) when the rate of heating was one-half degree per minute. The appearance of droplets on the upper wall of the tube, and the beginning of sintering of the mass of the specimen could be observed at about 77°. Placed in a bath at 80° and heated at a rate of 5° per minute, specimens melted at 85–87.5° (corr.). No difference in melting behavior was observed between specimens in ordinary soft glass tubes and in tubes made from Pyrex stock previously cleaned with chromic acid.

The aqueous layer, after benzene extraction of atebrin base, was of a distinct yellow color which was found to be almost completely removed by repeated chloroform extraction. To estimate the amount of this water-soluble matter, 1.000 Gm. of the commercial salt was dissolved in 15 cc. of warm water, 2 cc. of 20% sodium hydroxide added, and the mixture extracted four times with 25, 20, 15 and 15 cc. portions of benzene. The combined extracts were washed with three 5-cc. portions of water which were combined and extracted twice with 3-cc. portions of benzene which, in turn, were separately washed with a 2-cc. portion of water. All water phases were combined and shaken with nine 40-cc. portions of chloroform. The aqueous phase at this point was but faintly colored. The residue from the filtered chloroform extract, after drying one-half hour at 100°, amounted to 11.7 mg. (1.2%) of a brownish resin which was completely soluble in a small volume of chloroform.

Chromatographic Examination.—The base was liberated from 30 Gm. of commercial salt as previously described, but the extraction was effected with chloroform in order that the chromatogram include chloroform-soluble material. The solvent was removed as before, and the residue taken up in 100 cc. of warm benzene. On standing overnight a small amount of yellow, powdery material precipitated. Another 100 cc. of benzene was added and the turbid solution passed through a 2.5 x 28 cm. column of about 125 Gm. of Merck Brockmann alumina. The flow rate was increased by a mild air pressure at the top of the tube. After passage of an additional 50 cc. of benzene the following chromatogram was observed in daylight:

3 mm.	yellowish brown
8 mm.	dirty yellow
1 mm.	brown
15 mm.	orange
Remainder	yellow (atebrin)

The effluent contained unadsorbed atebrin. After development with 300–400 cc. of U. S. P. chloroform the appearance of the chromatogram was as follows:

3 mm.	gray
6 mm.	yellow
1 mm.	brownish
20 mm.	yellow
thin indistinct band	
2 mm.	yellow
1 mm.	brownish
25 mm.	almost white
20 mm.	pale orange
125 mm.	pale yellow
50 mm.	almost white
ca. 0.5	orange-brown
25 mm.	yellow (atebrin)

Because of the possibility that substances adsorbed more strongly than atebrin from a benzene solution might have their positions reversed with respect to that substance in the presence of chloroform, the atebrin residue from the combined benzene and chloroform effluents was dissolved in benzene and rechromatographed on a like column. After developing with benzene a 2-mm. brown band was apparent at the top of the column, the remainder of which appeared to be the uniform yellow of atebrin. The top layer was mechanically removed and the atebrin eluted from the rest of the adsorbent with ethanol. The residue from the combined eluate and benzene effluent was recrystallized from ethanol-water and dried as previously described. The product consisted of small prismatic forms, the optical crystallographic properties of which have been described recently by Keenan (3). Its melting point behavior did not differ from that of unchromatographed material.

Anal. Calcd. for $C_{23}H_{30}ClN_3O \cdot H_2O$: Cl, 8.48; loss on drying, 4.31; anhydrous base, 95.69. Found: Cl[3], 8.47, 8.51; loss on drying[4], 4.36, 4.26; anhydrous base[5], 95.5, 95.1.

When specimens were placed over calcium chloride, and the system evacuated to a pressure of less than 1 mm. at 30°, droplets of dehydrated material were observed in about one-half hour. After two hours a pasty mixture of hydrate and noncrystalline base was present.

A Dean and Stark distillation of dry toluene and a 10-Gm. sample yielded 0.41 cc. of water: n_D^{25}, 1.3320; b. p. (Siwoloboff), 100°.

Since the quantity of alumina used in chromatographing was sufficient to accommodate only more strongly adsorbed impurities and part of the atebrin, the possibility remained that weakly adsorbed substances might be present in the purified product. About 40 Gm. of alumina per gram of atebrin was necessary to explore this possibility. To one-half gram of purified base 50 cc. of benzene was added, and the solution dehydrated in an all-glass apparatus by distilling off 40 cc. of solvent. The residual liquid was passed into a 1 x 28-cm. column and developed with about 50 cc. of benzene (dried by distillation). In daylight the following chromatogram was observed:

25.5 cm.	yellow (atebrin)
1.5 cm.	almost white
1 cm.	white

[3] By G. Robert Clark, Cosmetic Division, U. S. Food and Drug Administration.

[4] Two hours at 100°.

[5] The U. S. P. (4) assay was used. Omission of the acetate buffer seems to exercise no significant effect on results obtained on the dihydrochloride. In applying the assay to the base, however, the buffer was of value in effecting solution of the sample.

In ultraviolet light the almost white zone appeared as a distinct yellow, while the white portion of the column was of a sky-blue color. The column was further developed until these two bands, representing traces of impurities, had passed from the column. The atebrin was eluted with methanol and recrystallized from ethanol-water. Rechromatographing of the product demonstrated the absence of the sky-blue component and the reappearance of yellow impurity which evidently was formed during the recrystallization.

Chromatographic examination of base not purified by the adsorption procedure showed that several strongly adsorbed substances were not eliminated by the benzene extraction and recrystallization.

Ultraviolet Irradiation of Atebrin.—One-half gram of chromatographically purified base was constantly rotated in a stoppered quartz tube for four hours while exposed to the unfiltered light of a quartz mercury-vapor lamp, delivering at the distance of 15 inches about 3300 microwatts between wave lengths of 1850 and 3150 Å. The irradiated material was somewhat darker in color and had an amine-like odor resembling that of old specimens of ephedrine base: m. p., 82–85° (slow) or 84.5–86.5° (rapid). After dehydration by benzene distillation, it yielded the following chromatogram in ultraviolet light on developing the 1 x 28-cm. column with benzene:

ca. 0.5 mm.	brown
20 mm.	yellow
thin orange band	
225 mm.	yellow (atebrin)
3 mm.	sky blue
10 mm.	yellow

SUMMARY

Atebrin base has been purified chromatographically and some properties of the crystalline substance have been observed.

REFERENCES

(1) Magidson, O. Yu., and Grigorovskiĭ, A. M., *Ber.*, 69 (1936), 396.
(2) Chelintsev, G. V., and Osetrova, E. D., *J. Gen. Chem.* (*U. S. S. R.*), 10 (1940), 1978.
(3) Keenan, G. L., THIS JOURNAL, 32 (1943), 248.
(4) "United States Pharmacopœia XII," Mack Printing Company, Easton, Pa., 1942, p. 383.

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Agaricic acid
3,4-Benzphenanthrene (0.2 Gm.)
Cytosine
Coenzyme II
Cytidylic acid
5-Ethyl-1,2-benzanthracene (0.2 Gm.)
4-Fluoro coumarin
4-Hydroxy coumarin
Guanylic acid
Isocitric acid
Unsym dichloro succinic acid or anhydride
Beta ionylidene acetaldehyde
Alpha keto glutamic acid
2-Methyl-3-(β hydroxy ethyl) pyridine
Phospho creatine
Ricin lipase
Rubrene
Triphenyl arsine
Triphenyl boron
Uridylic acid
Yellow enzyme (200 mg.)

CORRECTION—August 1944, pp. 225 and 245, "The U. S. P. Collaborative Cat Assays for Digitalis" by C. I. Bliss, and "The U. S. P. Collaborative Digitalis Study Using Frogs," by Lloyd C. Miller. A footnote should have been included in both of these papers to read as follows: "The publication of this report has been made possible by a contribution from the Board of Trustees of the United States Pharmacopœial Convention to THIS JOURNAL to cover the cost of printing."